

Risk Factors for Vertebral Fractures and Bone Loss after Denosumab Discontinuation: A Real-World Observational Study.

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1. Abstract

Background: Denosumab discontinuation without subsequent bisphosphonates (BPs) is associated with bone loss and multiple vertebral fractures.

Objective: Identifying risk factors for bone loss and vertebral fractures after denosumab discontinuation.

Methods: This retrospective study measured the outcome of 219 women with osteoporosis who discontinued denosumab treatment and received subsequent treatment with zoledronate, other BPs or a selective estrogen receptor modulator (SERM), or no therapy. Fracture rate, longitudinal bone mineral density (BMD) changes and bone turnover markers (BTMs) within 2 years after denosumab discontinuation were analysed. Linear regression analysis evaluated loss of BMD and age, BMI (kg/m²), denosumab treatment duration, pre-treatment, prior fracture state, baseline T-scores, use of glucocorticoids or aromatase inhibitors and BMD gains under denosumab therapy.

Results: 171 women received zoledronate after denosumab discontinuation, 26 had no subsequent treatment and 22 received other therapies (other BPs or a SERM). Zoledronate was associated with the fewest vertebral fractures (hazard ratio 0.16, p=0.02) and all subsequent therapies retained BMD at all sites to some extent. Higher BMD loss was associated with younger age, lower BMI, longer denosumab treatment, lack of prior antiresorptive treatment and BMD gain under denosumab treatment. BTM levels correlated with denosumab treatment duration and bone loss at the total hip, but not the lumbar spine.

Conclusions: Compared to no subsequent therapy, zoledronate was associated with fewer vertebral fractures after denosumab. Further, BMD loss depended on denosumab treatment duration, age, prior BP therapy and BMD gain under denosumab therapy, whereas BTM levels were associated with bone loss at the total hip and denosumab treatment duration.

2. Introduction

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3 Denosumab, a monoclonal antibody against the receptor activator of nuclear kB ligand
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5 (RANKL), is a potent antiresorptive agent commonly prescribed for the treatment of
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7 postmenopausal osteoporosis or the prevention of fractures and bone loss in patients with
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9 hormone ablative therapy. Treatment for up to 10 years results in continued gains in lumbar
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11 spine and total hip bone mineral density (BMD) without reaching a therapeutic plateau;
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13 further, the safety profile remains stable and the fracture incidence low¹⁻⁵. In contrast to
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15 bisphosphonates (BPs), denosumab does not incorporate into the bone matrix and therefore its
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17 effects are reversible when therapy is discontinued. After discontinuation of denosumab, bone
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19 turnover markers increase to above pre-treatment values, a response described as the ‘rebound
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21 phenomenon’⁶. If no subsequent therapy is administered, BMD gained during treatment is
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23 rapidly lost, and reaches baseline values within 12-24 months after the last denosumab
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25 injection^{6,7}. Furthermore, multiple spontaneous vertebral fractures have been reported⁸⁻¹⁴. To
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27 prevent bone loss and fractures after denosumab discontinuation, subsequent therapy with
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29 BPs has been recommended¹⁵⁻¹⁷. However, there is only limited evidence about the optimal
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31 subsequent regimen, and therefore many ongoing studies are currently addressing this
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33 question. Also, it remains unclear which patients are at risk for subsequent fractures and
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35 extensive bone loss after denosumab discontinuation.
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44 We recently reported 120 postmenopausal women who received 2 to 5 years of denosumab
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46 treatment, followed by a single infusion of zoledronate 6 months after the last denosumab
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48 injection¹⁸. At 2.5-year follow-up after denosumab cessation, fracture incidence was low: 1.1
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50 per 100 patient-years for vertebral fractures and 1.6 for non-vertebral fractures. Still, on
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52 average, 36% of BMD gained at the lumbar spine and 50% of that gained at the total hip was
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54 lost upon denosumab discontinuation, with a large range in the overall population. Since
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56 performing that study, we have included another 99 women (in particular women with longer
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denosumab treatment durations of 5 years or more). In the present study of now 219 patients,
we aimed to identify possible risk factors for vertebral fractures and bone loss after
denosumab discontinuation, and to determine how these events, as well as bone turnover,
were impacted by different subsequent therapies. Also, we compared the long-term outcome
of patients who received denosumab for at least 5 years with those treated for shorter periods.

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3. Methods

3.1. Setting and Outcome

This study, named ‘ProOff’ (Prolia Off-treatment), was a monocentric observational study of prospectively enrolled patients at OsteoRheuma Bern, Switzerland. The aim of this retrospective analysis was to identify possible risk factors for vertebral fractures and bone loss after denosumab discontinuation. The primary endpoint was the vertebral fracture rate after denosumab discontinuation with either a different subsequent treatment or no subsequent treatment. The secondary endpoints were the evolution of BMD and BTM and the identification of risk factors for vertebral fractures and/or bone loss after denosumab discontinuation. The off-treatment phase was defined as beginning 6 months after the last denosumab injection, and fractures were taken into account if they occurred within 24 months off-treatment (30 months post-injection).

3.2. Study Population

The patients reviewed in this retrospective study were treated with denosumab and evaluated by DXA between August 1, 2010, and November 30, 2019. Women who received ≥ 2 denosumab injections, who underwent DXA and VFA evaluation on the days of the first and last denosumab injections and who had at least one follow-up visit with DXA and VFA evaluation ≥ 12 months after the last denosumab injection were eligible for the study.

Patients underwent dual-energy X-ray absorptiometry (DXA) and vertebral fracture assessment (VFA) every 2 years after initiating denosumab therapy, according to established guidelines of pharmacological therapy in osteoporosis^{19,20}. Denosumab was usually discontinued if there was sufficient BMD gain, bone density nearing osteopenia (T-score at lumbar spine and total hip ≥ -2.5 SD) or low fracture risk (e.g., stopping co-treatment with

1 glucocorticoids or aromatase inhibitors). In some rare cases there were other reasons for
2 discontinuation (e.g., dental procedures, adverse effects).
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5 In most cases, zoledronate was chosen as subsequent treatment, but some patients (e.g., those
6 who had a history of an acute phase reaction due to zoledronate or those with a high risk for
7 breast cancer) received other BPs or SERMs. A few patients did not follow our advice and
8 decided against any therapy after denosumab discontinuation.
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14 BMD at the lumbar spine (L1-L4), total hip and femoral neck was measured before starting
15 denosumab (DXA1), at the last denosumab injection (DXA2) and at 1-4 years after the last
16 denosumab injection (DXA3), always including a VFA with standardized screening for
17 morphometric vertebral fractures ²¹. Clinically or morphometrically diagnosed vertebral
18 fractures were confirmed by MRI and/or lateral X-rays of the thoracolumbar spine.
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28 In some patients, a fourth DXA was performed at 4-6 years after denosumab discontinuation
29 (DXA4). Hologic Delphi S/N 70197 C or GE Lunar Prodigy Pro “Full” JBO/557-C devices
30 were used for measuring DXA and VFA, with a least significant change of 0.025 g/cm² for
31 the lumbar spine and 0.035g/cm² for the total hip. All measurements in each patient were
32 performed using the same device. All patients were asked about clinical fractures at the
33 DXA1, DXA2, DXA3 and DXA4 time points. If possible, C-terminal telopeptide of type I
34 collagen (CTX) and/or N-terminal propeptide of type 1 procollagen (P1NP) concentrations
35 were recorded at the DXA3 time point. The normal reference range of CTX concentration in
36 postmenopausal women was 0.06-0.50 ng/ml, while that of P1NP concentration was 15-59
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53 The study protocol was reviewed and approved by the local ethical committee (swissethics,
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55 related data.
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3.3. Statistical analysis

To investigate the impact of medical treatment on vertebral fractures within 30 months after the last denosumab injection, we used Cox regression (time to event) to account for observation periods shorter than 30 months. Events later than 30 months after denosumab discontinuation were right-censored from the analysis. If the time to fracture was unknown (i.e., for morphometrically diagnosed fractures), we assumed a duration of 30 months. We also took into account multiple vertebral fractures using Cox regression with multiple-failure data. We accounted for missing data among BMD gain or loss measurements by using multiple imputations with the T-score at DXA1 (first denosumab injection), DXA2 (last denosumab injection), DXA3 (median 26 months post-injection), and BMD gain or loss at all locations in the model to create 25 imputed datasets based on chained equations. We used linear regression to investigate the impact of the treatment on bone loss at time DXA3 as a percentage of DXA2 in three different locations (lumbar spine, total hip and femoral neck). We also included patient characteristics and treatment details as covariates in the linear model, and corrected the model for different time intervals between denosumab discontinuation and DXA3. In a subgroup of patients, CTX (n=53) and P1NP (n=59) were measured at DXA3, and these values were analysed as additional secondary endpoint without imputation. In patients with a fourth DXA measurement at 4-6 years after denosumab discontinuation, we used linear regression to compare the effect of 5-year versus 2.5-year denosumab treatment duration on BMD.

To investigate whether the duration of denosumab treatment had an impact on CTX and P1NP, we performed linear regression with adjustment for subsequent treatment regimen, patient age, change of hip density from DXA2 to DXA3, prior therapy (yes/no) and time interval between last denosumab injection and measurement of BTMs (performed at the same time as DXA3). Continuous variables are shown as mean (95% CI) or median [\pm Interquartile

1 Range, IR], and differences between treatment groups were tested using ANOVA or the
2 Kruskal-Wallis test, as appropriate. Categorical variables are shown as number (%), and their
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4 differences were tested using Fisher's exact test for binary variables or the chi-squared test
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6 otherwise. All analyses were carried out using Stata 16 (Stata Corporation, College Station,
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4. Results

Between August 1, 2010 (the date of Prolia® (denosumab) approval in Switzerland) and November 30, 2019, 859 patients received ≥ 1 dose of denosumab and were evaluated by DXA and VFA in our rheumatology department (Figure 1).

Of the 859 patients, 558 had ongoing treatment with denosumab and 219 discontinued denosumab. 45 of the 859 patients were lost to follow-up (4 changed physicians, 4 died and 37 had no contact with our institution for >3 years). Due to missing DXAs or insufficient technical quality, male gender or administration of only 1 denosumab injection, 37 patients were excluded.

Of the 219 women who discontinued denosumab and were included in this study, 171 received subsequent treatment with a single zoledronate infusion at 6-7 months after the last denosumab injection, 22 received subsequent treatment with other BPs (ibandronate po/iv, n=6; alendronate, n=10) or a selective estrogen receptor modulator (SERM; n=6) started 5 months after the last denosumab injection and 26 patients had no subsequent therapy (Figure 1). All patients received calcium and vitamin D supplementation.

4.1. Baseline characteristics

Age distribution, body mass index (BMI, kg/m²), prevalent vertebral and non-vertebral fractures, prior treatment with antiresorptive agents and duration of denosumab treatment are shown in Table 1, with little evidence of differences between groups (although aromatase inhibitors were used more commonly in women who did not receive a subsequent therapy after denosumab discontinuation). In addition, a comparison of included (n=219) and excluded (n=37) patients reveals no significant differences (data not shown). All women were Caucasian. 28 women received aromatase inhibitors and 21 received glucocorticoids during denosumab treatment. 9 patients received 2-3 denosumab injections (4%), 151 received 4-6

(69%), 21 received 7-9 (10%) and 38 received ≥ 10 (17%). 85 patients had a history of prevalent vertebral fractures (39%). Under denosumab therapy, two patients suffered fragility fractures (one vertebral and one non-vertebral fracture).

4.2. Vertebral and non-vertebral fractures after denosumab discontinuation

A total of 12 patients sustained vertebral and/or non-vertebral fractures within 30 months after the last denosumab injection (14 vertebral fractures in 8 patients and 6 peripheral fractures in 4 patients). Three patients without subsequent treatment therapy had asymptomatic morphometric vertebral fractures. Three peripheral fractures were associated with heavy trauma and were not considered to be osteoporotic fractures. The clinical characteristics of patients who sustained fractures after denosumab discontinuation are summarised in Table 2.

Multiple vertebral fractures were only observed in the group without subsequent treatment.

Compared to patients with no subsequent therapy (reference), zoledronate was associated with fewer vertebral fractures (hazard ratio [HR] 0.16, $p=0.002$), whereas other medications (SERMs and other BPs) demonstrated no significant difference (HR 1.07, $p=0.94$) (Table 3).

A direct comparison of zoledronate and other therapies yielded a HR of 0.16 for zoledronate ($p=0.04$). However, because there were only a few events, these results should be interpreted with caution.

In a univariable regression model, the factor with the strongest association with vertebral fractures, and hereby the only potential risk factor for vertebral fractures was a history of prior vertebral fractures (before denosumab was begun), with a HR of 3.84 [0.92 to 16.6] and a p -value of 0.06. Due to the small number of events (8 patients with vertebral fractures), no multivariate model could be performed ²².

4.3. BMD loss after denosumab discontinuation according to subsequent treatment

1 DXA2 was performed when the last denosumab injection was administered, while DXA3 was
2 performed 26 months (median) after the last injection (range 12-47 months, interquartile
3 range [IR] 20 to 30 months). During the off-treatment period, the mean BMD loss at all sites
4 was significantly different depending on the subsequent therapy or lack thereof. Patients who
5 received either zoledronate or BP/SERM after denosumab discontinuation demonstrated a
6 significantly lower decrease of BMD at all sites compared to patients who received no
7 treatment (Table 4). When analysing only treated patients, no difference was found between
8 patients with zoledronate and those with other BPs or SERM. Due to different subgroup sizes,
9 however, these results have to be interpreted carefully. Absolute BMD changes and T-scores
10 in all three subgroups are shown in Suppl. Table 1.
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24 *4.4. Risk factors for decrease in BMD after denosumab discontinuation*

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27 Simple linear regression analysis was performed in all patients, and, as a sensitivity analysis,
28 only in patients with subsequent off-treatment therapy, with percentage BMD loss between
29 DXA2 and DXA3 at the lumbar spine, total hip or femoral neck as the dependent variable.
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31 Independent variables were age, BMI (kg/m²), number of denosumab injections (and because
32 of dichotomous distribution and inclusion of few patients with 2 or 3 denosumab injections,
33 also with subgroups with >5 or ≤5 denosumab injections), baseline T-scores at the lumbar
34 spine, total hip and femoral neck, percentage increase of BMD during denosumab treatment at
35 all sites, concomitant therapy with glucocorticoids or aromatase inhibitors, prior
36 antiresorptive therapy, prevalent vertebral fractures and time interval of last injection to
37 follow-up DXA3. The results of the multivariable model, along with the coefficients (± 95%
38 CI) and p-values, are presented in Table 5. Younger age, lower BMI, longer denosumab
39 therapy and lack of prior antiresorptive treatment (before denosumab therapy) were
40 associated with increased BMD loss at the lumbar spine and/or total hip upon denosumab
41 discontinuation. Also, loss of BMD upon denosumab discontinuation at all sites was
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1 associated with gain of BMD under denosumab treatment at each anatomic site; this strong
2 association ($p < 0.005$) was even observed when the BMD gains and losses were corrected for
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4 treatment duration. In the univariate analysis (data not shown), use of aromatase inhibitors
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6 was associated with bone loss at the total hip. This was not observed in the multivariate
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8 model, indicating that the finding was likely due to confounding (perhaps because these
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10 patients were younger).
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14 As a sensitivity analysis, we used this same multivariable model in patients who received
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16 subsequent therapy after denosumab discontinuation ($n=193$). No relevant differences in the
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18 results were found, indicating that the associations were present in all treatment groups and
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20 were not primarily driven by the group that did not receive a subsequent therapy.
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23 24 25 *4.5. Long-term follow-up in patients treated with denosumab for 2.5 versus 5 years*

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27 In terms of denosumab treatment history, the largest subgroup of patients received 5
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29 denosumab injections and subsequent treatment with a single infusion of zoledronate (44%).
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31 Of these 96 patients, 43 already underwent DXA4, meaning a second DXA after denosumab
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33 discontinuation (4-6 years after the last denosumab injection). Between DXA3 and DXA4, 24
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35 of these 43 patients received BP therapy (a second infusion of zoledronate in most cases).
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37 However, 19 (44%) patients had no further treatment after the first zoledronate infusion, and
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39 we compared the patients with 2.5 years denosumab therapy duration (and subsequent
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41 treatment with BPs for 1-2 years) with the patients who were treated with denosumab for 5
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43 years and subsequently received BP therapy (in most cases with zoledronate) for 1 year
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45 ($n=41$). These 2 subgroups did not differ in baseline characteristics (age, BMI, baseline T-
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47 scores, prior therapy and prevalent fractures) (Suppl. Table 2). The longitudinal percent
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49 changes at the lumbar spine, total hip and femoral neck of these patients in comparison with
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51 patients with 5-year denosumab therapy and 1 year subsequent BP treatment are depicted in
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Figure 2. After 5 years, there was a significant difference of BMD change and T-scores compared to baseline at all sites (changes in T-scores adjusted for baseline: p-value <0.001 for lumbar spine, p=0.006 for total hip and p=0.028 for femoral neck), but after 6-8 years, percentage BMD changes and T-scores compared to baseline were similar in both groups (p-value 0.52 for lumbar spine, 0.48 for total hip and 0.91 for femoral neck).

4.6. Bone turnover markers after denosumab discontinuation

At the DXA3 time point, CTX levels were measured in 53 patients and P1NP levels were assessed in 59 patients (41 patients had both CTX and P1NP measurements). All analyses with BTM levels were corrected for the time interval between the last denosumab injection and measurement of BTMs.

The levels did not differ according to the subsequent therapy received after denosumab discontinuation (p-value 0.46 for CTX, 0.62 for P1NP). The median CTX concentrations were as follows: in patients with zoledronate (n=40), 0.38 ng/ml (IR 0.28 to 0.80); in those with other BPs/SERMs (n=7), 0.33 ng/ml (IR 0.18 to 0.44); and in those with no subsequent treatment therapy (n=6), 0.41 ng/ml (IR 0.39 to 0.47). The median P1NP levels were as follows: in patients with zoledronate (n=44), 50 ng/ml (IR 34 to 64); in those with BPs/SERMs (n=9), 31 ng/ml (IR 30 to 72); and in those with no subsequent therapy (n=6), 60 ng/ml (IR 46 to 65).

High CTX levels were significantly associated with longer denosumab treatment duration (p=0.006) and greater BMD loss at the total hip (p<0.001). Figure 3 shows the CTX and P1NP levels in patients with 5 denosumab injections versus patients with 6 or more denosumab injections (no measurements of BTM were obtained in patients with 4 or fewer denosumab injections). In a multivariate model with correction for the time interval since denosumab discontinuation, these levels were significantly different (CTX p=0.03, P1NP

p=0.028). CTX levels were not associated with the type of subsequent therapy (zoledronate versus BPs/SERMs) or with prior BP therapy. The coefficients and CIs of all variables in the multivariable model were the same as those in the univariable models, indicating no interactions. Associations of the variables in the prediction model of P1NP showed the same patterns as for CTX, except that age was negatively associated with P1NP.

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5. Discussion

5.1. BMD retention and vertebral fracture rate

The efficacy of subsequent BP treatment to preserve BMD gains after denosumab discontinuation has been examined in different studies and case series, with heterogeneous results. In some reports, subsequent treatment with zoledronate was largely able to prevent loss of gained BMD^{18,23–26}, but less optimistic results have also been demonstrated^{27–29}.

Similarly, BMD retention achieved by administering oral BP after denosumab discontinuation ranged from small BMD losses to complete reversal of the gains of denosumab therapy^{12,24,30}.

Most of our patients (78%) received zoledronate after discontinuation of denosumab, and they exhibited significantly higher BMD retention at all sites compared to patients without subsequent therapy. Also patients with other BPs or SERMs showed a significant retention of BMD at all sites. Patients whose subsequent therapy consisted of zoledronate versus other BPs or SERMs showed no significant difference regarding bone loss upon denosumab discontinuation. However, preliminary data (abstract) from a retrospective review of 94 patients showed reasonable efficacy for both oral and intravenous BPs regarding bone loss after denosumab discontinuation³¹.

A small case series reported that a single infusion of zoledronate may not be effective in preventing bone loss following denosumab when bone turnover is still suppressed²⁷. For this reason, many current guidelines propose administering a BP only when bone turnover markers start to rise³². However, both in our study population and in a prospective trial²⁵, zoledronate was also effective when administered beginning 6 months (\pm 3 weeks) after the last denosumab injection. Delayed denosumab administration results in bone loss³³, and therefore delayed administration of therapy following denosumab discontinuation is probably also disadvantageous. Recently published results from a randomized study showed no significant differences in BMD retention between patients who received zoledronate at 6

1 versus 9 months after the last denosumab injection and a third group in which the precise time
2 of administration depended on BTM levels showed no significant differences in BMD
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4 retention between these 3 groups, but BMD was lower when infusions were administered later
5 than the 6 months, especially at the hip. Further, 2 vertebral fractures occurred in the group in
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7 which zoledronate was given 9 months after the last denosumab injection ²⁹. Thus, a position
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9 statement from the European Calcified Tissue Society (ECTS) recommends an antiresorptive
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11 treatment initiated 6 months after the final denosumab injection ³⁴.
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17 A prospective, placebo-controlled trial demonstrated that zoledronate was able to prevent
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19 vertebral fractures after denosumab discontinuation ²⁵. In our observational study, the fracture
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21 rate in patients who received a subsequent therapy with BPs or SERMs was low and was
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23 comparable to other reported data ^{14,25,35}. Multiple vertebral fractures were rare and were only
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25 observed in patients without subsequent treatment. We found that zoledronate was negatively
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27 associated with vertebral fractures when administered as subsequent therapy after denosumab
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29 discontinuation, whereas other BPs (ibandronate or alendronate) or SERMs showed no
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31 significant difference in HR for vertebral fractures compared to the patient group with no off-
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33 treatment therapy. However, due to the small number of events and possible bias, this
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35 outcome has to be interpreted with caution.
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42 *5.2. Risk factors for fractures and bone loss after denosumab*

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45 Little is known about risk factors that may affect the response to BP treatment upon
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47 denosumab cessation. As already shown in the FREEDOM extension trial, prevalent vertebral
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49 fractures are a risk factor for new vertebral fractures ¹⁴. Our study also confirms earlier
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51 reports that prior treatment with BPs prevents loss of BMD to a greater extent than if no pre-
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53 treatment is administered ^{36,37}.
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1 Interestingly, we observed that younger age and longer duration of denosumab treatment
2 (>2.5 y) were associated with a pronounced loss of BMD upon denosumab discontinuation. It
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4 is well known that a single dose of denosumab, and perhaps even a second one³⁰, causes no
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6 rebound phenomenon^{36,38}. While increasing the denosumab treatment duration to >1 year was
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8 initially not thought to cause increased rebound with higher BTM levels and pronounced
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10 BMD loss, this possibility has recently been discussed²⁹. In contrast, other studies did not
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12 demonstrate an association between the duration of denosumab treatment and either BMD
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14 response^{12,25} or the incidence of vertebral fractures¹⁴. Thus, it has been suggested that
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16 patients at high risk for osteoporotic fractures and those with osteoporotic BMD levels should
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18 continue treatment with denosumab for up to 10 years, until the outcomes of controlled
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20 clinical trials investigating optimal post-treatment BP regimens become available¹⁵. Based on
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22 our findings, such a long duration of denosumab therapy may be an unfavourable option
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24 because we not only identified an association between number of denosumab injections and
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26 loss of BMD upon denosumab discontinuation, but also demonstrated that in long-term
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28 follow-up of up to 8 years, there was no significant difference in BMD gain or T-scores at any
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30 site compared to baseline in patients who were treated with denosumab for 2-3 years versus 5
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32 years (both with subsequent BP treatment).
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41 Baseline T-scores at all locations were not associated with bone loss upon denosumab
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43 discontinuation, but larger BMD gains at all sites during denosumab treatment correlated with
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45 increased loss of BMD upon discontinuation. Notably, this association was independent of
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47 denosumab treatment duration and was also found in patients who received all 5 denosumab
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49 injections. We observed this same phenomenon in our previous study¹⁸, and similar findings
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51 were reported in the FREEDOM trial and in patients with 12 months of denosumab therapy in
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53 the DAPS study (Denosumab Adherence Preference Satisfaction)^{14,30}. This might be an
54
55 aftereffect of initial bone remodelling potential before denosumab therapy is started: Patients
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1 with higher bone turnover may gain more BMD under therapy, but also lose a greater
2 percentage of this gained BMD upon discontinuation³⁹. Thus, these patients should perhaps
3
4 be treated more aggressively with BPs after denosumab withdrawal.
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7 Denosumab has been approved for the prevention of osteoporosis in patients who have
8
9 undergone hormone ablative therapy as well as those with glucocorticoid-induced
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11 osteoporosis^{40,41}. In a few case series, women receiving aromatase inhibitors were
12
13 considered to be potentially at risk of vertebral fractures upon denosumab discontinuation
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5.3. Bone Turnover Markers

Many current denosumab discontinuation strategies recommend regularly measuring BTM
levels and modifying subsequent treatment accordingly^{15,32}. We found an association
between higher BTM levels after denosumab discontinuation and both bone loss at the total
hip and longer treatment duration. Interestingly, the type of off-treatment therapy
(zoledronate, other BPs or SERMs) had no influence on CTX and/or P1NP concentrations,
although different BP therapies show different intensities of bone turnover suppression⁴⁴⁻⁴⁶.
Therefore, this finding should be interpreted with caution. But an association between
denosumab treatment duration and CTX levels was previously reported in a small
observational study of women with aromatase inhibitor therapy⁴², and may be explained by
long-term inhibition of osteoclast differentiation under denosumab treatment leading to a
larger pool of osteoclast precursors, all of which enter differentiation upon denosumab
withdrawal. This theory is consistent with our observation of increased BMD loss with longer
denosumab treatment duration. Notably, in that analysis we corrected for the time interval
between last denosumab dose and follow-up since this interval differed among patients in our

1 study population, and bone turnover after withdrawal of denosumab is known to change over
2 time ⁶.
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4 5 *5.5. Limitations* 6

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8 Our retrospective study has several limitations, including selection bias due to heterogeneous
9 treatment modalities. Regarding fracture rates, the results have to be interpreted with caution
10 due to the low number of events. Also, because DXA2 was performed around the time of the
11 last denosumab injection and not 6 months later (when the medication effect ends), the
12 amount of BMD gained under denosumab therapy and the loss upon discontinuation was
13 probably underestimated. Finally, while follow-up DXA was performed 1-4 years after
14 denosumab discontinuation in the overall population, >90% were evaluated within 3 years. In
15 addition, it has previously been shown that bone loss after denosumab discontinuation occurs
16 mainly within 2 years ^{18,26}, so we do not believe that the range of DXA assessments biased
17 our results.
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31 32 33 *5.6. Conclusions* 34

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36 We conclude that the fracture rate after denosumab discontinuation remained low in patients
37 with subsequent antiresorptive treatment. Multiple vertebral fractures were only observed in
38 patients without subsequent therapy. Zoledronate was associated with a lower incidence of
39 vertebral fractures within 24 months after denosumab discontinuation. BMD retention did not
40 differ significantly according to treatment strategy after denosumab discontinuation
41 (zoledronate, other BPs or SERMs). Younger age and longer duration of denosumab therapy
42 were significantly associated with higher BMD loss after denosumab discontinuation. Thus, if
43 denosumab is to be discontinued, special attention should be paid to early postmenopausal
44 patients and those who have been treated with denosumab for long periods (>3 years). These
45 patients require more intensive monitoring and treatment than older patients with shorter
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1 treatment durations. Also, patients with prevalent fractures should be treated and monitored
2 more intensively because they are at particular risk for new vertebral fractures after
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4 denosumab discontinuation. Future randomized controlled trials will help to determine the
5
6 optimal denosumab therapy duration and the appropriate subsequent treatment strategy.
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9 **Author contributions and Acknowledgements**

10
11 All authors were involved in drafting the article or critically revising it for important
12
13 intellectual content, and all authors approved the final version to be submitted for publication.
14
15

16
17 Study conception and design: JE, TL, SR. Acquisition of data: TL, JE, US, HRZ. Analysis
18
19 and interpretation of data: JE, BG, TL, SR. BG and JE take responsibility for the integrity of
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21 the data analysis.
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7. Legends

Figure 1 Flow chart of the Observational Study

Flow chart of the inclusion of patients who discontinued denosumab treatment and were followed up with DXA and VFA 1-4 years later. Dmab: denosumab, BPs: bisphosphonates, SERM: selective estrogen receptor modulator.

Table 1 Patient characteristics and further descriptive items

Dmab: denosumab, BPs: bisphosphonates, SERM: selective estrogen receptor modulator.

Table 2 Clinical characteristics of patients with vertebral or non-vertebral fractures after denosumab discontinuation

Dmab: denosumab, BPs: bisphosphonates, SERM: selective estrogen receptor modulator, ZOL: zoledronate, IBN: ibandronate, VFx: vertebral fractures, LS: lumbar spine, TH: total hip, FN: femoral neck, na: not available (asymptomatic fractures with morphometric diagnosis)

Table 3 Vertebral fractures after denosumab discontinuation

Dmab: denosumab, BPs: bisphosphonates, SERM: selective estrogen receptor modulator.

Table 4 BMD loss in relation to subsequent treatment

BPs: bisphosphonates, SERM: selective estrogen receptor modulator.

Suppl. Table 1 Absolute BMD changes and T-scores

BPs: bisphosphonates, SERM: selective estrogen receptor modulator.

Table 5 Risk factors for bone loss after denosumab discontinuation

1 Dmab: denosumab, LS: lumbar spine, TH: total hip, FN: femoral neck. Vfx: vertebral
2 fracture(s), AI: aromatase inhibitor
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6 **Suppl. Table 2 Characteristics of Patients with long-term follow-up**

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8 Dmab: denosumab, BPs: bisphosphonates, LS: lumbar spine, TH: total hip, FN: femoral neck,
9 AI: aromatase inhibitor.
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16 **Figure 2 Long-term follow-up**

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18 Longitudinal percent changes from baseline of LS-BMD (A), TH-BMD (C) and FN-BMD (E)
19 in patients with 2.5 years` denosumab treatment (and 1-2 years of subsequent treatment with
20 BPs) versus 5 years of denosumab treatment (and 1 year of subsequent treatment with BPs).
21 Symbols represent mean \pm CI. Dmab: denosumab, BP: bisphosphonate, LS: lumbar spine,
22 TH: total hip, FN: femoral neck.
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30 **Figure 3 Bone turnover markers and treatment duration**

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32 Boxplots represent CTX levels (A) and P1NP levels (B) in patients with 5 versus >6
33 denosumab injections. The median CTX level in patients with 5 denosumab injections was
34 0.34 ng/l (0.23, 0.43), and that in patients with > 6 denosumab injections 0.40 ng/ml (0.31,
35 0.56). The median P1NP level in patients with 5 denosumab injections was 43 ng/l (31, 56),
36 and that in patients with > 6 denosumab injections was 57 ng/ml (39, 80). Dmab: denosumab.
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Table 1: Patient characteristics and further descriptive items

	No subsequent treatment (n = 26)	Zoledronate (n = 171)	Other therapy (BPs or SERM) (n = 22)	p-values
Age at incl.	65 ± 7.9	66 ± 7.8	67 ± 8.0	0.72
BMI at incl.	24 ± 4.1	24 ± 3.8	23 ± 3.7	0.60
Number of Dmab injections	5.0 [5.0 to 7.0]	5.0 [5.0 to 7.0]	5.0 [5.0 to 8.0]	0.53
>5 Dmab injections	10 (38%)	57 (33%)	8 (36%)	0.85
Any prior therapy	8 (31%)	70 (41%)	7 (32%)	0.52
Use of aromatase inhibitor	7 (27%)	17 (10%)	4 (18%)	0.04
Use of steroids	3 (10%)	14 (8.1%)	4 (17%)	0.35
Prevalent fractures				0.67
No	10 (38%)	74 (43%)	7 (32%)	
Peripheral	8 (31%)	46 (27%)	5 (23%)	
Vertebral	8 (31%)	51 (30%)	10 (45%)	0.32

Variables are presented as mean (95% CI), median [lq to uq] or n (%), as appropriate

Table 2: Clinical Characteristics of 13 Patients with Vertebral or Non-Vertebral Fractures after Denosumab Discontinuation

Vertebral Fractures										
	Site of Fx	Off treatment	Age at inclusion	Baseline T-Score (LS/TH)	Prevalent VFxs	Prior BP treatment	Time on Dmab (years)	BDM change under Dmab treatment (LS/TH)	BMD change after Dmab stop (LS/TH)	Last injection to Fx (months)
Pat. 1	L3	ZOL	74	-1.7/-1.3	yes	yes	2.5	+5.4%	-4.7%	18
Pat. 2	T12	ZOL	63	-2.7/-3.3	no	yes	2.5	-1.0%	+3.4%	24
Pat. 3	T11 & L2	None	75	na/-2.1	yes	no	2	+2.2%	-4.8%	22
Pat. 4	T9,T10,T12, L3,L4	None	81	-2.3/-2.5	yes	no	2	na	na	na
Pat. 5	L1	ZOL	75	-2.9/-1.2	yes	yes	5	+7.5%	-3.5%	12
Pat. 6	L1	SERM	56	-2.3/-1.8	no	yes	2.5	+0.3%	-3.9%	14
Pat. 7	L1	IBN	70	-2.7/-2.8	yes	no	4	+12.7%	-3.6%	na
Pat. 8	T8, T11	None	60	-2.4/-1.3	no	no	2.5	+4.3%	-7.0%	na
								+4.1%/+1.2%	na	na
								+9.8%/+8.7%	-12.1%	na
									-12.4%	
Non-Vertebral Fractures¹										
Pat. 1	Calcaneus	ZOL	62	-1.9/-1.5	yes	yes	2.5	+7.8%	-4.8%	18
Pat. 2	Radius	ZOL	67	-3.6/-0.9	no	yes	5	+0.5%	+4.4%	10
Pat. 3	Humerus& Metatarsal	ZOL	57	-2.7/-2.3	no	no	3	+17.3%	-2.9%	18
Pat. 4	FN & Hip	None	68	-1.6/-2.6	no	no	2.5	+6.1%	-1.4%	17
								+11.3%	-5.6%/	
								+3.4%	-1.9%	
								+11.6%	na	
								+2.8%		

¹ Three traumatic fractures are not listed

Table 3: Vertebral fractures after denosumab discontinuation

A. Association of occurrence of vertebral fracture within 24 months after Denosumab discontinuation (30 months post-injection) with the subsequent therapy

Endpoint	Treatment	# Pat.	Hazard ratio (95% CI)	P-value
Entire cohort				
Time to vertebral fracture within 24 months	No subsequent therapy	3	Reference	
	Zoledronate	3	0.16 (0.03 to 0.77)	0.023
	Other therapy (BP or SERM)	2	1.07 (0.18 to 6.42)	0.944
Number of fractures*	No subsequent therapy	9	Reference	
	Zoledronate	3	0.06 (0.01 to 0.26)	<0.001
	Other therapy (BP or SERM)	2	0.34 (0.06 to 1.89)	0.217
Only treated patients				
Time to vertebral fracture within 24 months	Other therapy (BP or SERM)	2	Reference	
	Zoledronate	3	0.16 (0.03 to 0.94)	0.042

Note that in three patients without subsequent therapy, time to vertebral fracture was unknown and fractures were diagnosed 26, 31 and 47 months after denosumab discontinuation, respectively. We heuristically assumed 24 months.

*Patients who received zoledronate, BPs or SERM did not present with multiple vertebral fractures during follow-up.

Table 4: BMD loss in relation to subsequent treatment

Associations of medical treatments with percentage loss of bone density between last denosumab injection (DXA2) and DXA3 at three different locations

Localisation	Treatment	Coefficient (95% CI)	P-value
Entire cohort			
Change at lumbar spine (%)	No subsequent therapy	Reference	
	Zoledronate	2.15 (0.34 to 3.96)	0.020
	Other therapy (BP or SERM)	2.88 (0.42 to 5.34)	0.022
Change at total hip (%)	No subsequent therapy	Reference	
	Zoledronate	2.86 (1.36 to 4.37)	<0.001
	Other therapy (BP or SERM)	2.04 (0.13 to 3.94)	0.037
Change at femoral neck (%)	No subsequent therapy	Reference	
	Zoledronate	4.34 (2.09 to 6.60)	<0.001
	Other therapy (BP or SERM)	3.49 (0.42 to 6.55)	0.026
Only treated patients			
Change at lumbar spine (%)	Other therapy (BP or SERM)	Reference	
	Zoledronate	-0.73 (-2.60 to 1.14)	0.441
Change at total hip (%)	Other therapy (BP or SERM)	Reference	
	Zoledronate	0.83 (-0.60 to 2.25)	0.254
Change at femoral neck (%)	Other therapy (BP or SERM)	Reference	
	Zoledronate	0.86 (-1.41 to 3.12)	0.457

Time Interval DXA2 to DXA3 (Median):

BP/SERM: 23 months; No off-treatment: 26 months; Zoledronate: 26 months

Table 5: Risk factors for Bone Loss after Denosumab Discontinuation

Multivariable associations between patient characteristics and change of bone density between DXA2 (last Denosumab injection) and DXA3 in % at different anatomic sites after multiple imputation

Covariate	At lumbar spine, %		At total hip, %		At femoral neck, %	
	Coefficient (95% CI)	P-value	Coefficient (95% CI)	P-value	Coefficient (95% CI)	P-value
Age per 10 years	1.12 (0.43 to 1.82)	0.002	0.64 (0.08 to 1.20)	0.026	0.62 (-0.26 to 1.49)	0.166
BMI at incl.	0.15 (0.00 to 0.30)	0.047	0.06 (-0.05 to 0.18)	0.283	0.07 (-0.11 to 0.25)	0.452
# of Dmab Injections	-0.34 (-0.61 to -0.06)	0.016	-0.29 (-0.50 to -0.08)	0.007	0.01 (-0.33 to 0.35)	0.965
>5 Dmab Injections	-1.24 (-2.44 to -0.03)	0.044	-1.16 (-2.12 to -0.21)	0.017	0.30 (-1.21 to 1.81)	0.693
Prior therapy*	-1.17 (-2.29 to -0.04)	0.043	-0.36 (-1.26 to 0.54)	0.428	-0.39 (-1.80 to 1.02)	0.586
Prior vFx	0.34 (-0.91 to 1.60)	0.588	0.07 (-0.92 to 1.06)	0.896	0.18 (-1.39 to 1.75)	0.819
Use of AI	0.81 (-1.03 to 2.65)	0.386	-1.29 (-2.78 to 0.20)	0.089	-1.48 (-3.79 to 0.83)	0.208
Use of Steroids	-0.19 (-2.07 to 1.69)	0.841	0.10 (-1.41 to 1.62)	0.894	-0.86 (-3.20 to 1.49)	0.473
T-Score LS DXA1	0.23 (-0.51 to 0.97)	0.543	0.30 (-0.30 to 0.90)	0.320	0.64 (-0.30 to 1.58)	0.182
T-Score TH DXA1	-0.24 (-1.53 to 1.05)	0.713	0.29 (-0.70 to 1.28)	0.562	1.21 (-0.41 to 2.83)	0.143
T-Score FN DXA1	0.56 (-0.68 to 1.81)	0.371	-0.08 (-1.05 to 0.89)	0.877	-1.10 (-2.75 to 0.55)	0.190
Change [‡] at LS	-0.17 (-0.29 to -0.04)	0.011				
Change [‡] at TH			-0.15 (-0.29 to -0.01)	0.042		
Change [‡] at FN					-0.35 (-0.50 to -0.19)	<0.001
Time interval**	-0.06 (-0.15 to 0.03)	0.179	-0.01 (-0.08 to 0.07)	0.890	-0.03 (-0.14 to 0.08)	0.576

*Antiresorptive therapy within 2 years before Dmab was started

‡ Change between DXA1 and DXA2

** Time Interval between last dmab injection and DXA3





