A Single Infusion of Zoledronate in Postmenopausal Women Following Denosumab Discontinuation Results in Partial Conservation of Bone Mass Gains.

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Author contributions

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1. Abstract

Discontinuation of denosumab is associated with a rapid return of bone mineral density (BMD) to baseline and an increased risk of multiple vertebral fractures. No subsequent treatment regimen has yet been established for preventing either loss of BMD or multiple vertebral fractures after denosumab discontinuation. The aim of this 8-year observational study was to investigate the effect of a single zoledronate infusion, administered 6 months after the last denosumab injection, on fracture occurrence and loss of BMD. We report on 120 women with postmenopausal osteoporosis who were treated with 60 mg denosumab every 6 months for 2 to 5 years (mean duration 3 years) and then 5 mg zoledronate 6 months after the last denosumab injection. All patients were evaluated clinically, by DXA and vertebral fracture assessment (VFA), before the first and after the last denosumab injection and at 2.5 years (median) after denosumab discontinuation. During this off-treatment period, 3 vertebral fractures (1.1 per 100 patient years) and 4 non-vertebral fractures (1.5 per 100 patient years) occurred. No patients developed multiple vertebral fractures. Sixty-six percent (CI: 57-75%) of BMD gained with denosumab was retained at the lumbar spine, and 49% (CI: 31-67%) at the total hip. There was no significant difference in the decrease of BMD between patients with BMD gains of >9% vs. <9% while treated with denosumab. Previous antiresorptive treatment or prevalent fractures had no impact on the decrease of BMD, and all bone loss occurred within the first 18 months after zoledronate infusion**.** In conclusion, a single infusion of 5 mg zoledronate after a 2 to 5-year denosumab treatment cycle retained more than half of the gained BMD and was not associated with multiple vertebral fractures, as reported in patients who discontinued denosumab without subsequent bisphosphonate treatment.

Key Words: Denosumab, Discontinuation, Fracture, Osteoporosis, Zoledronate

2. Introduction

Denosumab is a monoclonal antibody against the receptor activator of nuclear kB ligand (RANKL) and is a potent antiresorptive agent commonly prescribed for treatment of postmenopausal osteoporosis. In contrast to bisphosphonates, denosumab does not incorporate into bone matrix and therefore its effects are reversible when therapy is discontinued. After termination of denosumab, BMD gained during treatment is rapidly lost and reaches baseline values within 12 months after the last denosumab injection [1,2]. Furthermore, multiple spontaneous vertebral fractures occurring 9-16 months after the last denosumab injection have recently been reported [3–7]; these are summarized in a review of 24 cases of multiple vertebral fractures [8]. Post-hoc analysis of the FREEDOM and FREEDOM extension trials showed no excessive vertebral fractures in women who discontinued denosumab after 2 to 5 doses, but the median off-treatment observation duration was only 8 months, and at least one-third of the patients had been started on an anti-osteoporotic treatment during this period [9,10]. The number of new vertebral fractures was 7.1 per 100 participant years, compared with 8.5 per 100 participant years in the placebo group [11].

The authors of these reports and case series all concluded that denosumab should not be discontinued without subsequent antiresorptive therapy. However, no post-denosumab regimen has yet been established. A working group of the European Society of Calcified Tissue (ESCT) and other experts in the field recommend switching to bisphosphonates 6-9 months after the last denosumab injection [7,12–14]. Thus, the complete loss of BMD can probably be prevented, but little is known about the incidence of vertebral fractures during subsequent bisphosphonate therapy. Also, it remains unclear which patients are at risk of (multiple) vertebral fractures or particularly high BMD loss after denosumab discontinuation.

We previously reported 22 cases of women with postmenopausal osteoporosis who received a single infusion of zoledronate after terminating denosumab treatment. None of the patients

experienced new vertebral fractures [15]. In the current retrospective observational study, we report the outcome of 120 women with postmenopausal osteoporosis who underwent the same sequential off-treatment protocol; we studied the incidence of new vertebral and non-vertebral fractures, the increase and decrease of BMD at the lumbar spine, total hip and femoral neck, and possible effects of prior bisphosphonate therapy and prevalent vertebral and non-vertebral fractures. Additionally, we analysed possible correlations between loss of BMD after denosumab discontinuation and age, body mass index (BMI) and increase of BMD during denosumab treatment.

3. Methods

OsteoRheuma Bern AG (ORB) is a medical practice with 4 resident rheumatologists who treat patients with osteoporosis within the regulations and restrictions of the Swiss health insurance system. When denosumab was first approved in Switzerland for treatment of women with postmenopausal osteoporosis in August 2010, it was already well known that a rebound of osteoclast activity with a rapid decrease of BMD occurred after termination of denosumab [2]. Physicians working at ORB therefore decided that if denosumab were to be discontinued, they would treat their patients with a single zoledronate infusion 6 months after the last denosumab injection. Patients were checked by 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 [16,17]. In cases of low fracture risk or sufficient BMD gain close to osteopenia (T-score at lumbar spine ≥ -2,0 SD) , the responsible physician and his patient usually decided to switch to zoledronate 6 months after the last denosumab injection. Otherwise, treatment with denosumab was continued for another 2 years.

When it became apparent that discontinuing denosumab was not only associated with a rapid loss of gained BMD but also with an increased risk of multiple vertebral fractures, the authors decided to retrospectively review the data of patients who had been treated with this approach.

Patients reviewed in this retrospective study were treated with denosumab and evaluated by DXA between August 1, 2010 and March 31, 2019. Women aged >48 years with postmenopausal osteoporosis who received >2 injections of 60 mg denosumab every 6 months and a single dose of 5 mg zoledronate 6 months after the last injection of denosumab were eligible for the study. After the single zoledronate infusion, no further anti-osteoporotic medication was administered, except for calcium and vitamin D supplementation.

BMD at the lumbar spine (L1-L4), total hip and femoral neck was measured before starting denosumab (DXA1), at the last denosumab injection (DXA2) and at 1 to 4 years after denosumab discontinuation (DXA3). Hologic Delphi S/N 70197 C or GE Lunar Prodigy Pro "Full" JBO/557-C devices were used for measuring DXA and VFA. All patients were asked about clinical fractures at the DXA1, DXA2 and DXA3 time points.

Where available, C-terminal telopeptide of type I collagen (CTX) and/or N-terminal propeptide of type 1 procollagen (P1NP) concentrations at DXA3 time points were recorded.

Patients without subsequent treatment after denosumab discontinuation were analysed regarding fracture rate and BMD changes at DXA1, 2 and 3.

The study protocol was reviewed and approved by the local ethical committee (swissethics, 2019-00008) and all women provided written informed consent for further use of their health-related data.

The Shapiro-Wilk test was used to test normal distribution. Comparisons of percentage decrease of BMD over time were made with a paired t-test. Comparison of the difference in mean percentage loss of BMD between patients with a small or large gain of BMD under denosumab treatment was made with an unpaired, 2-sided Student's t-test. Comparisons of the subgroups (prior bisphosphonate treatment, prevalent fractures and time delay between last denosumab injection and follow-up DXA) were tested with repeated measures analysis of variance with Tukey's post hoc test and Kruskal-Wallis-test or analysed by fitting a mixed model in case of sporadic missing values. Correlations were tested with Spearman's test or Pearson's test, as appropriate. Data are shown as mean ± 95% confidence interval (CI) or median ± interquartile range (IQ). Statistical significance was established at $p<0.05$. Statistical analysis was performed with Graph Pad Prism Software Version 8.02.

4. Results

Between August 1, 2010 (date of the approval of Prolia® (denosumab) in Switzerland) and March 31, 2019, 9023 patients were reviewed, of whom 836 received ≥1 dose of denosumab and were measured by DXA and VFA in our rheumatology department (Figure 1).

Of the 836 patients, 461 had ongoing treatment with denosumab and 336 discontinued denosumab. Thirty-nine of the 836 patients were lost to follow-up (4 changed physicians, 3 died and 32 had no contact with ORB for >3 years).

Of the 336 patients who discontinued denosumab, 276 received subsequent treatment with a single zoledronate infusion at 6 months after the last denosumab injection (Figure 1). One hundred twenty-eight of the 276 patients did not complete follow-up by DXA3 and 24 patients did not undergo 3 DXAs. For instance, patients with a short duration of denosumab treatment (1-3 injections) did not undergo DXA at the last injection (DXA2); they had just 2 DXA measurements (DXA1 and DXA3). Thus, we did not have information about loss of BMD upon denosumab discontinuation. Two patients had invalid DXA measurements (insufficient technical quality). Finally, 120 postmenopausal women with 2- to 5 years of denosumab treatment and 1 zoledronate infusion were included in our study. Exclusion criteria were as follows: male gender, premenopausal status and ≥1 DXA measurement missing.

Baseline characteristics

Age distribution, BMI, prevalent vertebral and non-vertebral fractures, prior treatment with antiresorptive agents and mean BMD T-scores at the lumbar spine, total hip and femoral neck are shown in Table 1. All patients were Caucasians. Nine patients received aromatase inhibitors and 9 received glucocorticoids prior to or during denosumab treatment. Ninety-

seven patients received 4-6 denosumab injections (81%), 11 received 7-9 denosumab injections (9%) and 12 received 10 denosumab injections (10%).

Vertebral fractures

Three patients developed symptomatic vertebral fractures between 1-3 years after the last denosumab injection. With a mean off-treatment interval of 26.9 months, we calculated an incidence of 1.1 per 100 patient years. Vertebral fracture analyses, which were routinely performed with all DXA scans, recorded no additional vertebral fractures. No patients exhibited multiple vertebral fractures.

Patient 1: A 74-year-old woman was diagnosed with osteoporosis 12 years before starting denosumab, with original T-scores of -2.8 SD (lumbar spine) and -1.5 SD (total hip). She had previously sustained an osteoporotic L4 fracture as well as a traumatic fracture of her humeral head. Early menopause was her only clinical risk factor. She was treated with oral and intravenous bisphosphonates for 5 years prior to being switched to denosumab. Baseline (DXA1) T-scores were -1.7 SD (lumbar spine) and -1.3 SD (total hip). Eighteen months after the fifth denosumab injection, a symptomatic, spontaneous L3 fracture occurred. Following 2.5 years of denosumab treatment, her BMD had increased by 5.4% at the lumbar spine and decreased by 1.0% at the total hip. Nineteen months after the fifth denosumab injection, her BMD had decreased by 4.7% at the lumbar spine and increased by 3.4% at the total hip.

Patient 2: A 63-year-old woman was diagnosed with postmenopausal osteoporosis 7 years before starting denosumab, with original T-scores of -2.4 SD (lumbar spine) and -3.2 SD (total hip). She had no prevalent fractures and no other clinical risk factors and was treated with bisphosphonates for 7 years before denosumab therapy was initiated. Two years after the fifth denosumab injection she sustained a symptomatic T12 fracture while coughing. Upon terminating 2.5 year treatment with denosumab, her BMD increased by 6.5% at the lumbar

spine and 2.2% at the total hip since starting denosumab treatment. Twenty-nine months after the last denosumab injection, her BMD had decreased by 2.2% at the lumbar spine and 4.8% at the total hip.

Patient 3: A 68-year-old woman was diagnosed with osteoporosis 5 months prior to baseline. She suffered spontaneous multiple vertebral fractures (T11, L1 and L4) and her risk factors for fragility fractures were early menopause and a family history of osteoporotic vertebral fractures. She was treated with oral bisphosphonates for 5 months prior to being switched to denosumab. Baseline (DXA1) T-scores were -2.0 SD (lumbar spine) and -1.6 SD (total hip). Three years after the last denosumab injection (7 injections) and subsequent to 1 infusion of zoledronate, a symptomatic worsening of her pre-existing L3 vertebral deformity occurred. This patient was initially lost to follow-up, but after she sustained a fracture, she was referred for a DXA scan and resumption of anti-osteoporotic treatment. After termination of denosumab treatment (DXA2), her BMD increased by 15.8% at the lumbar spine and 1.5% at the total hip. Forty months after the seventh denosumab injection (DXA3), BMD had decreased by 2.0% at the lumbar spine and by 1.6% at the total hip.

In summary, all 3 patients sustained only a single vertebral fracture. They had all been previously treated with bisphosphonates. The first patient had a single prevalent vertebral fracture and the third patient sustained multiple vertebral fractures prior to initiation of denosumab treatment.

Non-vertebral fractures

Four patients developed peripheral fractures (1.4 per 100 patient years). One patient sustained a traumatic fracture of the os pubis in the context of a massive fall while skiing 7 months after the fifth denosumab injection. The second patient suffered a traumatic humerus fracture 2.5 years after discontinuation of denosumab. The third patient was a 62-year-old woman who

experienced a spontaneous fracture of the calcaneus 18 months after the fifth denosumab injection. In this patient, BMD increased by 7.8% at the lumbar spine and 0.5% at the total hip between DXA 1 and DXA 2, and decreased by 4.8% at the lumbar spine and increased by 4.4% at the total hip at DXA3. The fourth patient was a 67-year-old woman who suffered a fracture of the distal radius in the context of a fall 4 months after the single zoledronate infusion. During 5 years of treatment with denosumab her BMD increased by 8.9% at the lumbar spine and 2.4% at the total hip, but decreased by 2.9% the lumbar spine and 1.4% at the total hip upon discontinuation of denosumab. CTX and P1NP levels 21 months after the last denosumab injection were 0.32 ng/ml and 52 ng/ml, respectively.

Increase and decrease of BMD

DXA1 was performed at denosumab treatment initiation, while DXA2 was performed when the last injection was administered. Treatment duration was 2-5 years, and BMD increased between DXA1 and DXA2 by 9.7% at the lumbar spine, 4.6% at the total hip and 3.5% at the femoral neck. Between DXA2 and DXA3, BMD decreased by 3.3% at the lumbar spine (+6.4% compared to baseline), 2.2% at the total hip (+2.4% compared to baseline) and 1.5% at the femoral neck (+2.0% compared to baseline). The longitudinal percent changes of BMD at the lumbar spine, total hip and femoral neck are depicted in Figure 2; decrease of BMD upon denosumab discontinuation was significant in all regions ($p< 0.0001$ at lumbar spine and total hip, $p<0.005$ at femoral neck). The percentage of retained BMD gain was 66% (CI: 57-75%) at the lumbar spine, 49% (CI: 31-67%) at the total hip and 57% (CI: 25-89%) at the femoral neck. DXA3 was performed 29 months (median; IQ: 23-30) after the last denosumab injection (with a single subsequent zoledronate infusion administered 6 months after the last denosumab injection), but with a wide range of 12-42 months. The frequency distribution of the monthly interval between DXA2 and DXA3 is shown in Figure 2.

In subgroups of patients whose BMD increased by <9% versus >9% at the lumbar spine while treated with denosumab, the lumbar spine BMD decreased by 4.1% in patients with a BMD gain of >9%, which was larger than the 2.7% decrease in patients with a BMD gain <9%. This difference did not reach statistical significance (p=0.051; mean difference-1.4%, CI: -2.9, +0.01%) (Figure 3). Furthermore, percentage increase of BMD was not significantly correlated with percentage decrease of BMD at the lumbar spine (R -0.18; p=0.07; CI: -0.4, +0.02) or total hip (R -0.15; p =0.12; CI: -0.3, +0.04), as shown in Figure 3. There was no correlation between percentage loss of BMD at the lumbar spine or total hip after terminating denosumab and either age or BMI (data not shown).

Chronology of BMD decrease

Assessments at DXA3 were performed between 12-42 months (median: 29 months) after the last denosumab injection. In subgroups of patients whose DXA3 took place within the first 12-23 months (n=30), 24-30 months (n=42) and 31-42 months (n=48) after the last denosumab injection, the percentage decrease of BMD did not differ significantly at the lumbar spine (p=0.1) or total hip (p=0.7), suggesting that all of the BMD loss after denosumab discontinuation and zoledronate infusion occurred within the first 18 months (24 months post-injection) (Figure 4).

Prior vertebral and non-vertebral fractures

Prior to the initiation of denosumab treatment, 52 patients (43%) had no prevalent fractures, 37 (31%) had prior non-vertebral fractures and 31 (26%) had ≥1 vertebral fracture (with or without an additional non-vertebral fracture). Percentage BMD decreases were not statistically different between patients with or without prior vertebral fractures or between patients with or without non-vertebral fractures (p= 0.5 for lumbar spine; p= 0.2 for total hip) (Figure 4).

Prior bisphosphonate treatment

Of the 120 patients, 57 (48%) had no prior treatment for osteoporosis before denosumab was initiated, except for calcium and vitamin D supplementation. Sixty-two (52%) patients had previously been treated with bisphosphonates, and 42 of these patients received bisphosphonates within 2 years before denosumab was initiated. One patient received teriparatide prior to denosumab treatment and this patient was not included in this subgroup analysis. With respect to percentage BMD loss after terminating denosumab treatment, there was no difference between the subgroups: patients with or without prior bisphosphonate treatment and patients with or without a drug holiday had a similar percentage decrease of lumbar spine BMD after terminating denosumab treatment ($p=0.23$). Regarding values at the total hip, patients with no prior bisphosphonate treatment had a significantly higher percentage increase of BMD during treatment with denosumab ($p<0.05$; mean difference +1.3%, CI: +0.4, +2.3) (Figure 4).

Patients without subsequent treatment after discontinuation of denosumab

In our study population of 836 patients treated with denosumab, we identified 28 patients without subsequent treatment after discontinuation of denosumab (Figure 1). Most of these patients stopped denosumab due to side effects or other health issues, for instance dental procedures. We could not use these patients as a control group because there were not enough complete data sets in this group (missing DXAs). Still, we recorded DXA1, DXA2 and DXA3 for 11 of the 28 patients. They received denosumab for 0.5 to 5 years, with a mean of 1.5 years. These 11 patients gained 8.1% of their baseline lumbar spine BMD (CI: +6.1%, +10.1%) under treatment with denosumab and lost 6.1% by 1-2 years after discontinuation (CI: -8.6%, -3.4%). At the total hip, BMD increased by 4.7% (CI: +3.2%, +6.3%) under treatment and decreased by 5.9% upon discontinuation (CI: -7.8%, -4.1%). Three of the 11

patients suffered fractures: 2 sustained multiple vertebral fractures (3 and 5 vertebrae affected), which occurred 18 months post-injection or which were diagnosed morphometrically 24 months post-injection. One patient had a spontaneous atraumatic fracture of the pelvis and femoral neck 11 months post-injection.

Bone turnover markers after denosumab discontinuation

We had not regularly obtained blood samples to determine the levels of bone turnover markers in our patients, but at the time of this study we did have data on CTX and/or P1NP concentrations in 27 patients; these data were obtained 1-4 years after the last denosumab injection and subsequent to a single infusion of zoledronate (Figure 5). None of the 27 patients sustained a vertebral fracture. The mean CTX concentration was 0.41 ng/ml (normal range for postmenopausal women: 0.06-0.50 ng/ml) and the mean P1NP concentration was 51.2 ng/ml (normal range: 15-59 ng/ml), as shown in Figure 5. The values are also shown in relation to the number of months between DXA2 (last denosumab injection) and DXA3 (1-4 years later and subsequent to a single zoledronate infusion). There was a significant negative correlation between the DXA2-DXA3 interval and P1NP concentration (R -0.58; $p < 0.005$; CI: -0.8, -0.2). No significant correlation was observed for CTX concentration (R -0.18; $p=0.47$; CI: -0.6, +0.3).

5. Discussion

Treatment with 60 mg denosumab every 6 months was associated with a reduction of fracture risk and a substantial increase of BMD, as reported in the FREEDOM Trial and its Extension [18]. However, in a phase 2 multidose trial and an extension of a phase 3 study a rapid BMD drop to baseline was observed within 12 months after terminating denosumab [1,2], and some patients sustained vertebral fractures [3–8] . Vertebral fractures after denosumab discontinuation occurred between 8-18 months after the last denosumab injection when no subsequent therapy was initiated, with an incidence of 7-15 per 100 patient years [10,11]. It is uncertain whether the increase in fracture rates after stopping denosumab occurs simply because rapid bone loss returns the patient to their pre-existing risk of fragility fractures, or whether it instead predisposes to a higher risk of fractures in the short term. Still, a few studies reported that denosumab discontinuation was associated with an increased risk of multiple vertebral fractures [7,10].

To prevent such fractures, subsequent bisphosphonate treatment has recently been recommended. However, no specific regimen has been established so far. Two years ago we reported the first case series of 22 patients who were treated with 5 denosumab injections and a single zoledronate infusion 6 months after the fifth denosumab injection, and who experienced no subsequent vertebral fractures [15]. In the present study of 120 patients with the same subsequent treatment protocol, the incidence of vertebral fractures was 1.1 per 100 patient years, and no patients sustained multiple vertebral fractures. A single infusion of zoledronate 6 months after the last denosumab injection was able to prevent the complete loss of BMD back to baseline, which occurs without subsequent treatment. Between 50-70% of BMD gained during denosumab treatment was retained at the lumbar spine as well as at the total hip.

Based on earlier reports, we assumed that prior treatment with bisphosphonates, especially without a drug holiday before initiating denosumab, would prevent loss of BMD to a greater extent than if no pre-treatment was administered [19,20]. We also expected patients with prevalent vertebral fractures to have a greater loss of BMD than patients without prevalent fractures, since patients in the FREEDOM extension trial who experienced prevalent vertebral fractures were at highest risk of new vertebral fractures after discontinuation of denosumab [10]. However, neither prior treatment with antiresorptive agents nor prevalent fractures had any influence on the decrease of BMD after discontinuing denosumab treatment.

Patients with a greater BMD increase at the spine (>9%) did not lose significantly more BMD upon discontinuation than patients with a lower BMD increase (<9%). The 3 patients who experienced a vertebral fracture did not lose more BMD than the overall average. We conclude that patients at the highest risk of vertebral fractures are not necessarily those who have gained or lost the most BMD. Also, the BMD increase under treatment with denosumab did not correlate with the decrease of BMD upon discontinuation, and there was no correlation of BMD decrease upon discontinuation with either age or BMI. Thus, it remains unclear which patients are at risk of rapid loss of BMD and vertebral fractures after discontinuation of denosumab.

The effects of zoledronate were previously evaluated in a case series of 6 women with postmenopausal osteoporosis treated with denosumab for 7 years in the FREEDOM and FREEDOM extension trials [21]. A single dose of 5 mg zoledronate was administered 6 months after the last dose of denosumab. There was a significant decrease in BMD at the lumbar spine and total hip 18-23 months later, with the lumbar spine BMD remaining significantly above the pre-treatment baseline and the total hip BMD not significantly differing from baseline. The authors suggest that this rather disappointing treatment effect may have been due to reduced skeletal uptake of zoledronate due to the strong suppression of bone

remodelling by denosumab. The current study, which is substantially larger, shows more promising results.

Another case series evaluated the effect of zoledronate, oral risedronate or placebo [22]. Women involved in the FRAME study (Fracture Study in Postmenopausal Women) were offered zoledronate, oral risedronate or no treatment after 1 year of romosozumab versus placebo, followed by 2 years of open-label denosumab. Eleven women chose to receive zoledronate as a single 5-mg infusion with a median delay of 65 days from the trial endpoint, 5 chose oral risedronate at a dosage of 35 mg per week and 3 chose no additional treatment. While women receiving no treatment retained 10-20% of gained BMD at the end of FRAME, women who chose zoledronate retained 73% at the lumbar spine and 87% at the total hip. Women who received risedronate demonstrated an intermediate decrease of BMD. These results regarding zoledronate resemble those of our study.

Discontinuation of denosumab is associated with an increase of bone turnover above pre-treatment levels, a phenomenon described as a "rebound effect" that is probably linked to the upregulation of osteoclastogenesis [1,23]. In this observational study, we had only limited information about bone turnover markers upon denosumab discontinuation, but we did have data from 27 patients obtained 1-4 years after the last denosumab injection and subsequent to a single infusion of zoledronate. CTX and P1NP concentrations were within the upper normal range, indicating newly active bone turnover that contrasts with the almost complete cessation of bone resorption during ongoing treatment with denosumab [1,24]. This may be interpreted as a "dangerous" rebound effect associated with an increased risk of vertebral fractures. However, given the fact that very few fractures occurred, we assume that these markers may indicate a gradual return to more physiological bone remodelling, as seen in patients without antiresorptive treatment or in those who terminate treatment with bisphosphonates and have a subsequent "drug holiday" of several years' duration [25]. The observation that P1NP

concentrations were higher when measured soon after denosumab discontinuation may contribute to this hypothesis.

We are well aware that this retrospective "real-world" study has many limitations, including the relatively low quality of its evidence compared to prospective (randomized) control studies. One main problem is possible selection bias: We discontinued denosumab treatment in patients who demonstrated BMD gain sufficient to achieve or almost achieve osteopenia. If this was not the case after 5-6 injections, denosumab was continued for another 2-3 years. We report here on patients who were treated for a relatively short period of time, namely 2-3 years, due to a satisfactory clinical course and an adequate gain of BMD. Only 19% of patients in our study population had longer treatment durations (more than 3 years). So far, no data have been published on patients treated for longer periods of time; we expect such data approximately 2 years from now. It has been suggested that a longer denosumab treatment duration reduces the effect of zoledronate regarding BMD retention after denosumab discontinuation [26], but this was not confirmed by McClung et al. [7] Moreover, no association between the duration of denosumab treatment and the incidence of vertebral fractures was reported [10]. Another limitation of this study was a minor change of selection criteria after analysing certain patients: We first intended to include only patients with a maximum treatment duration of 3 years, but later added an additional 23 patients with a longer treatment duration in order to reduce selection bias. The results were not substantially changed by the inclusion of these 23 patients.

We did not define a control group, but did collect complete data sets (DXA1-3) from 11 of 28 patients who did not adhere to our treatment recommendations and who therefore received no subsequent therapy after discontinuation of denosumab. As expected, BMD of the lumbar spine and total hip decreased to baseline. The loss of 6% BMD at the lumbar spine in these 11 patients was the same as that observed over 12 months by Miller, Bone and McClung [1,7,27].

Furthermore, 2 of these 11 patients sustained multiple vertebral fractures, while 1 experienced atraumatic pelvic and femoral neck fractures. These results are consistent with a previous report of multiple vertebral fractures that occurred after denosumab discontinuation without subsequent antiresorptive therapy [8].

We conclude that a single infusion of 5 mg zoledronate after 2- to 5-year denosumab treatment retained more than half of the gained BMD and was not associated with multiple vertebral fractures, as reported in patients who discontinued denosumab without subsequent bisphosphonate treatment. This pragmatic therapeutic regime may be a promising step in identifying sequential long-term treatment strategies for osteoporosis. Nevertheless, each patient requires an individualized surveillance and treatment plan after denosumab discontinuation, including BMD assessment, evaluation of bone turnover markers and consideration of individual clinical risk factors, in particular prevalent fragility fractures.

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7. Legends

Figure 1 Flow chart of the observational study.

Flow chart of the selection of women who discontinued denosumab treatment and 6 months later received a single infusion of 5 mg zoledronate.

Table 1 Baseline characteristics.

Figure 2 Longitudinal percent changes of BMD of all patients.

Longitudinal percent changes (compared to baseline) of BMD at the lumbar spine (A), total hip (B) and femoral neck (C). Symbols represent Mean ± CI. DXA1: Initiation of denosumab; DXA2: Termination of denosumab (last injection); DXA3: 2.5 y (median) after last denosumab injection and single infusion of zoledronate. (D) Frequency distribution of the interval in months between the last denosumab injection (DXA2) and follow-up DXA (DXA3). *Dmab=Denosumab, Zol=Zoledronate.*

Figure 3 Association between BMD increase under denosumab treatment and BMD decrease after discontinuation.

(A) Percentage increase and decrease of lumbar spine BMD in patients with a gain of lumbar spine BMD >9% versus <9% (-4.1% versus -2.7% BMD loss after discontinuation of denosumab and infusion of zoledronate). Boxplots represent Median ± IQ. (B, C) Correlation between percentage increase and decrease of BMD at the lumbar spine (R -0.18) or total hip (R -0.15).

Figure 4 Influence of prior treatment, prevalent fractures and follow-up interval on BMD decrease after denosumab discontinuation.

Longitudinal percent changes of BMD (compared to baseline) at the lumbar spine (A) and total hip (B) depending on the monthly interval between DXA2 and DXA3. Longitudinal percent changes (compared to baseline) of BMD at the lumbar spine (C) and total hip (D) depending on prevalent fracture state. Longitudinal percent changes (compared to baseline) of lumbar spine BMD (E) or total hip BMD (F) depending on prior treatment with bisphosphonates. One patient received teriparatide before switching to denosumab and was

excluded from this analysis. Boxplots represent Median ± IQ. *BP=Bisphosphonates.* DXA2: Termination of denosumab (last injection); DXA3: 2.5 y (median) after last denosumab injection and single infusion of zoledronate.

Figure 5 Bone turnover markers after denosumab discontinuation.

CTX (A) and P1NP (C) concentrations in 27 patients obtained 1-4 years after the last denosumab injection and subsequent to a single infusion of zoledronate, represented as Median ± IQ. (B) and (D) show the individual values in terms of months between DXA2 (last denosumab injection) and DXA3 (1-4 years later and subsequent to a single zoledronate infusion). The mean CTX concentration was 0.41 ng/ml (normal range for postmenopausal women: 0.06-0.50 ng/ml) and the mean P1NP concentration was 51.2 ng/ml (normal 15-59 ng/ml). *Dmab=Denosumab.*

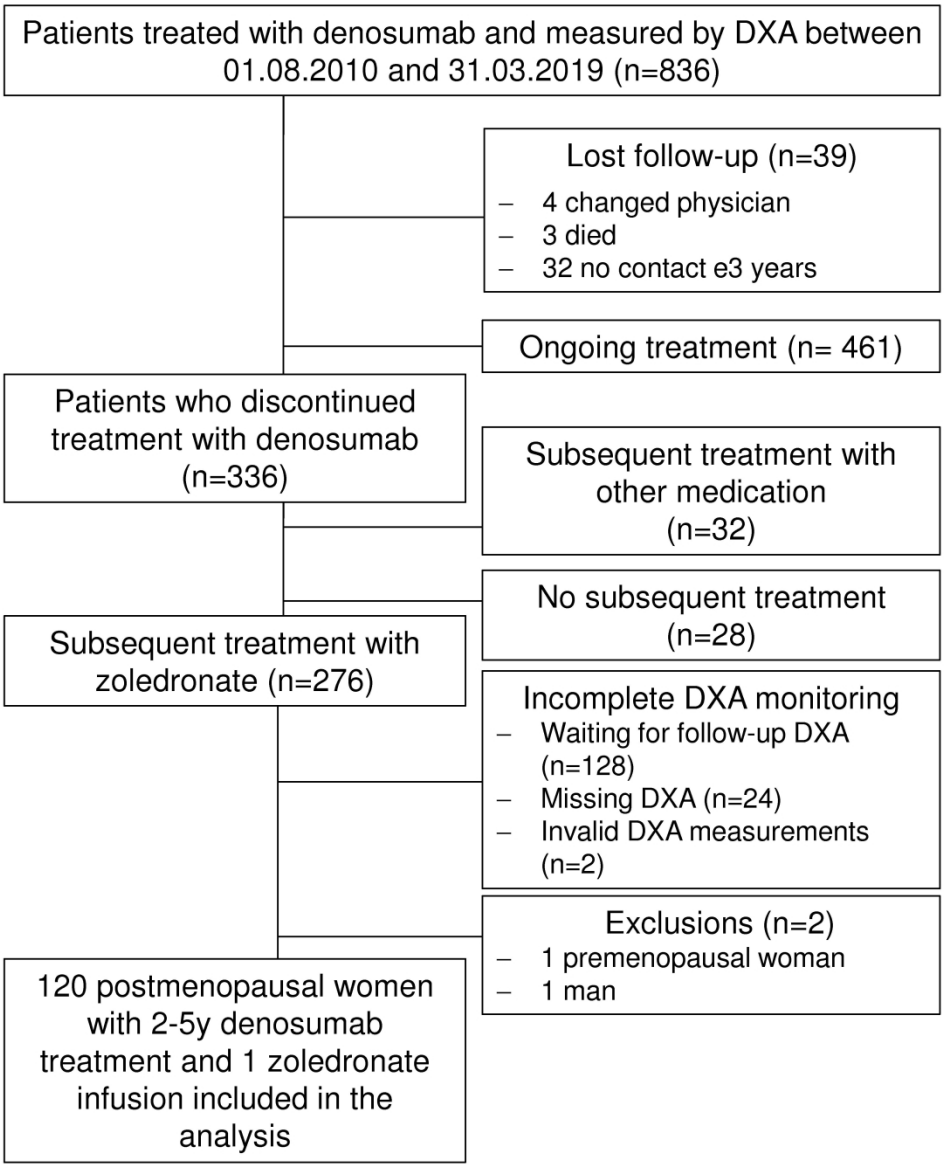

Table 1 Baseline characteristics

Characteristics	Study Population (n=120)
Age, years[1]	65.6 (49-85)
BMI (kg/m^2)[2]	24.1(23.4-24.8)
Prior antiresorptive treatment	62 patients (52%)
Prior vertebral fractures	31 (26%)
Prior non-vertebral fractures	37 (30%)
T-score lumbar spine[2]	-2.3 (-2.1, -2.5)
T-score total hip[2]	-1.7 (-1.6, -1.9)
T-score femoral neck[2]	-2.1 (-2.0, -2.2)

Table 2. Clinical Characteristics of 5 Patients who Sustained Vertebral or Non-Vertebral Fractures after Denosumab Discontinuation

	Site of Fx	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 discontinuation (LS/TH)	Last injection to Fx (months)
Vertebral Fractures									
Pat. 1	L3	74	-1.7/-1.3	yes	yes	2.5	+5.4% -1.0%	-4.7% +3.4%	18
Pat. 2	T12	63	-2.4/-3.2	no	yes	2.5	+6.5% +2.2%	-2.2% -4.8%	24
Pat. 3	L3	68	-2.0/-1.6	yes	yes	3.5	+15.8% +1.5%	-2.0% -1.6%	36
Non-Vertebral Fractures[1]									
Pat. 1	Calcaneus	62	-1.9/-1.5	yes	yes	2.5	+7.8% +0.5%	-4.8% -4.4%	18
Pat. 2	Radius	67	-3.0/-0.8	no	yes	5	+8.9% +2.4%	-2.9% -1.4%	10

[1] Two traumatic peripheral fractures are not included