The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. XX, No. XX, 1–8
doi:10.1210/clinem/dgab321
Clinical Research Article

Clinical Research Article

The Duration of Denosumab Treatment and the Efficacy of Zoledronate to Preserve Bone Mineral Density After Its Discontinuation

Polyzois Makras,[1,*] Natasha M. Appelman-Dijkstra,[2,*] Socrates E. Papapoulos,[2] Sandra van Wissen,[2] Elizabeth M. Winter,[2] Stergios A. Polyzos,[3] Maria P. Yavropoulou,[1,4] and Athanasios D. Anastasilakis[5]

[1]Department of Endocrinology and Diabetes and Department of Medical Research, 251 Hellenic Air Force & VA General Hospital, 11525, Athens, Greece; [2]Center for Bone Quality, Department of Internal Medicine, section Endocrinology, Leiden University Medical Center, 9600 2300 RC, Leiden, The Netherlands; [3]First Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece; [4]Endocrinology Unit, 1st Department of Propaedeutic and Internal Medicine, School of Medicine, National and Kapodistrian University of Athens, 10679, Athens, Greece; and [5]Department of Endocrinology, 424 General Military Hospital, 56429, Thessaloniki, Greece;

ORCiD numbers: 0000-0002-3231-3357 (P. Makras); 0000-0001-5035-127X (N. M. Appelman-Dijkstra); 0000-0002-0119-1588 (E. M. Winter); 0000-0002-9624-6296 (A. D. Anastasilakis).

*Authors share first co-authorship.

Abbreviations: BMD, bone mineral density; BMI, body mass index; BTM, bone turnover marker; CTX, C-terminal telopeptide of type 1 collagen; CV, coefficient of variation; Dmab, denosumab; DXA, dual-energy x-ray absorptiometry; ECTS, European Calcified Tissue Society; FN, femoral neck; LS, lumbar spine; P1NP, procollagen type 1 N-terminal propeptide; ZOL, zoledronate.

Received: 23 February 2021; Editorial Decision: 1 May 2021; First Published Online: 12 May 2021; Corrected and Typeset: 7 September 2021.

Abstract

Context: Zoledronate is used to prevent bone loss following denosumab discontinuation but its efficacy differs among studies.

Objective: To test if the duration of denosumab treatment affects the efficacy of subsequent zoledronate infusion.

Methods: This multicenter, prospective cohort study, conducted at 2 Greek and 1 Dutch bone centers, included 47 postmenopausal women (n = 47) who received a single zoledronate infusion 6 months after the last denosumab injection and then were followed for 1 year. Twenty-seven women received ≤ 6 denosumab injections (≤ 6 Group) and 20 received > 6 denosumab injections (> 6 Group). The main outcome measure was changes in lumbar spine (LS) bone mineral density (BMD).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Results: At 12 months LS-BMD values were maintained in the ≤ 6 Group (0.98 ± 0.10 to 0.99 ± 0.9 g/cm^2, $P = 0.409$) but decreased significantly in the > 6 Group (1.0 ± 0.11 to 0.93 ± 0.12 g/cm^2, $P < 0.001$). The percent change of LS-BMD of the ≤ 6 Group (+1.0%) was significantly different ($P < 0.001$) from the change of the > 6 Group (–7.0%). In the whole cohort, the duration of denosumab treatment was negatively correlated with the percentage change of LS-BMD ($r_s = –0.669$, $P < 0.001$) but not with the change of femoral neck (FN)-BMD. Bone turnover markers increased in all patients 6 months following zoledronate administration with no difference between the 2 groups.
Conclusion: The duration of denosumab treatment significantly affects the efficacy of subsequent zoledronate infusion to maintain BMD gains. Frequent follow-up of patients treated with denosumab longer than 3 years is advisable as additional therapeutic interventions may be needed.

Key Words: bone mineral density, bone turnover markers, denosumab, postmenopausal osteoporosis, zoledronate

In patients with osteoporosis who are discontinuing denosumab (Dmab) therapy, an increase in bone turnover above pretreatment values, resulting in rapid decrease of bone mineral density (BMD), is typically observed (1, 2). To prevent this "rebound phenomenon," it is currently recommended that patients who are stopping Dmab should be treated with bisphosphonates (3) and intravenous zoledronate (ZOL) is the most widely studied in randomized controlled trials and observational studies (4-11). There are, however, differences in reported efficacy of ZOL to maintain denosumab-induced gains in BMD, with some studies showing preservation of BMD after 1 year in the majority of treated patients (4, 5, 12), while in other studies the effect was only partial (6, 7, 10). These results raise questions about potential patient-related and/or treatment-related determinants of the response to ZOL. Two clinically important determinants are the timing of the ZOL infusion and the period of Dmab administration before its discontinuation. While a 6-month interval between the Dmab injection and the ZOL infusion is currently accepted as optimal timing independently of the levels of bone turnover markers (3), the effect of duration of Dmab therapy and its relation to the changes of bone turnover and BMD remain to be fully elucidated.

We addressed these questions in treatment-naïve women with postmenopausal osteoporosis who were treated with denosumab for 1.0 to 5.5 years and received a single ZOL infusion 6 months after the last Dmab injection.

Patients and Methods

AfterDmab (Zoledronic Acid to Maintain Bone Mass After Denosumab Discontinuation) was a 2-year parallel assignment, open label, multicenter, randomized, efficacy study (NCT02499237) (4). According to the study protocol, treatment-naïve postmenopausal women with osteoporosis received Dmab until reaching osteopenic BMD values at the hip or spine, and then 1 of the 2 arms of the study received an intravenous infusion of ZOL 5 mg 6 months after the last Dmab injection (ZOL arm) and was followed for 2 years. A total of 27 patients were initially included in the ZOL arm of the study and both the initial results as well as the results of a third-year follow-up of the study extension have been recently published (4, 5).

Twenty-five additional patients fulfilling the AfterDmab study inclusion and exclusion criteria were administered a single ZOL infusion 6 months following the last Dmab injection. Five out of the 25 additional patients were lost to follow-up (2 died, 1 developed breast cancer and did not follow the protocol's visits, and 2 retracted their consent); the 20 remaining patients were prospectively followed according to the AfterDmab study protocol in the Endocrinology outpatient clinics of: the 424 General Military Hospital, Thessaloniki, Greece; the 251 Hellenic Air Force & VA General Hospital, Athens, Greece; and the Leiden University Medical Center, Leiden, The Netherlands.

The total cohort of 47 patients, including the 27 patients of the AfterDmab ZOL arm and the 20 prospectively followed additional patients, was retrospectively divided into 2 groups according to the median number (6.0) of Dmab injections: the ≤ 6 Group (≤ 6 Dmab injections or ≤ 3 years of Dmab treatment) and the > 6 Group (7 or more Dmab injections or > 3 years of Dmab treatment), and were analyzed accordingly. All patients had received cholecalciferol 800 IU/day and calcium carbonate 500 mg twice daily and had normal serum 25-hydroxyvitamin D and calcium concentrations both at the time of ZOL administration and throughout the 12 months of follow-up. No patient received a second ZOL infusion. The protocol for treatment and follow-up was approved by the Medical Ethical Committees of all 3 hospitals; all AfterDmab patients signed an informed

consent according to institutional requirements. The additional patients were originally part of an opting-out protocol which changed to opting in and patients still under care have signed informed consent according to institutional requirements.

At baseline and 12 months areal BMD was measured by dual energy x-ray absorptiometry (DXA) at the lumbar spine (LS; L1-L4) and femoral neck (FN) (Lunar Corporation, Madison, WI, USA) and radiographs of the spine were performed at the same time points. Morning fasting blood samples were obtained from all participants right before and at 6 and 12 months following ZOL infusion for the measurement of serum procollagen type 1 N-terminal propeptide (P1NP), and C-terminal telopeptide of type 1 collagen (CTX), as previously described (4). Specifically, the samples of the 27 patients from the AfterDmab study were measured in a single batch by electrochemiluminescence immunoassay (ECLIA) on a Cobase 411 analyzer (Roche Diagnostics, Mannheim, Germany) (P1NP intraassay coefficient of variation [CV] ≤ 2.3%, interassay CV ≤ 3.0%; CTX intraassay CV ≤ 2.5%, interassay CV ≤ 4.6%); the samples from the additional 20 patients were not measured in a single batch but on a daily basis using the same assay and either the Cobase 411 analyzer or the E-170 system (Roche BV, Woerden, The Netherlands; assay variation for CTX 2.5% and for P1NP 3%).

Treatment Outcomes

We aimed to compare the 1-year effect of ZOL infusion given 6 months following the last Dmab injection among patients with a history of either ≤ 3 years of Dmab treatment (the ≤ 6 Group) or > 3 years of Dmab treatment (the > 6 Group).

The primary endpoint of this analysis was the difference in LS-BMD changes between the 2 groups from baseline to 12 months. Secondary endpoints included: the difference in FN-BMD changes between the 2 groups from baseline to 12 months; the relationship between the duration of Dmab treatment and BMD changes at the LS and FN; and the differences in serum bone turnover marker levels between the 2 groups throughout the 12 months of follow-up. The incidence of new vertebral fractures (clinical and morphometric) and other fragility fractures were exploratory endpoints.

Statistical Analysis

Data of baseline characteristics are summarized by mean ± SD unless stated otherwise. The Shapiro-Wilk test was used to test the normality of distribution of continuous variables. The Levene's test was used to assess the homogeneity of variance. To compare continuous variables (absolute values) between 2 independent groups, independent sample T-test or Mann-Whitney test were performed, depending on the normal or nonnormal distribution of data, respectively. In case of more than 2 repeated measures, repeated measures analysis of variance (ANOVA) or Friedman test was used. In case of statistically significant trend, multiple pairwise comparisons were performed with Bonferroni post hoc correction. Spearman's (r_s) coefficient of correlation was used for bivariate correlations between continuous variables. Analysis was intention-to-treat. A 2-sided P value of < 0.05 was considered statistically significant in all tests. Statistical analysis was performed with IBM SPSS Statistics, version 25.

Results

Forty-seven postmenopausal women (mean age 65.7 ± 9.2 years) were included in the present analysis: 27 patients in the ≤ 6 Group and 20 patients in the > 6 Group. Eleven patients had received exactly 6 Dmab injections and were all included in the ≤ 6 Group. The patients in the > 6 Group had lower body mass index (BMI) and more prevalent fractures at Dmab discontinuation, while no other statistical differences between the 2 groups were observed at baseline (Table 1).

Changes in LS-BMD and FN-BMD in both groups 1 year after the ZOL infusion are shown in Fig. 1 and Table 2. Compared with baseline, LS-BMD did not change at 12 months in the ≤ 6 Group. However, in the > 6 Group LS-BMD significantly decreased. Regarding the primary endpoint of the study, the percentage change of LS-BMD of the ≤ 6 Group (+1.0%) was significantly different (P < 0.001) compared with the relevant change of the > 6 Group (−7.0%), although the absolute BMD values did not differ between groups either at baseline or 12 months (Table 2). FN-BMD did not change in the ≤ 6 Group but decreased significantly in the > 6 Group. Similar to LS-BMD, the absolute FN-BMD values did not differ between groups at baseline and 12 months, and this was also the case with the changes as the 1.26% increase of the ≤ 6 Group was not different (P = 0.079) than the 2.56% decrease of the > 6 Group. The duration of Dmab treatment was negatively and significantly correlated with the percentage change of LS-BMD (r_s = −0.669, P < 0.001) but not with that of FN-BMD (r_s = −0.187, P = 0.241) (Fig. 2).

At 12 months, 2 patients were classified as having osteoporosis both at the LS and FN in the ≤ 6 Group and 2 in the > 6 Group.

Bone Turnover Markers

At study entry (6 months after the last Dmab injection) serum P1NP levels were above the upper limit of the postmenopausal reference range (76 ng/mL) in 2 patients, 1 in the ≤ 6 Group and 1 in the > 6 Group, while in 1 patient from the ≤ 6 Group these were above the premenopausal values (56 ng/mL); 1 patient of the ≤ 6 Group had serum CTX values above the premenopausal reference range (0.573 ng/mL) (Fig. 3). The ZOL infusion was followed by a significant increasing trend in serum CTX and P1NP values during the 12 months following ZOL infusion in both groups (Table 2 and Fig. 3).

In the ≤ 6 Group serum CTX levels rose significantly at 12 months; however, in only 3 patients values were above the premenopausal range at that time point (Fig. 3). The change between baseline and 6 months was not significant in contrast with the change between 6 months and 12 months (Table 2). In the > 6 Group the CTX changes were not significant either at 6 or 12 months, respectively (Table 2), while CTX levels were above the upper limit of the premenopausal range at 12 months in only 1 patient (Fig. 3).

In both groups, serum P1NP levels significantly increased at 12 months. However, changes in either group occurred after 6 months from ZOL administration (Table 2). In the ≤ 6 Group 2 patients had values above the premenopausal range at 6 months; at 12 months, 1 patient had values above the upper limit of the premenopausal range and 4 above the postmenopausal range (Fig. 3). In the > 6 Group, 1 patient had values above the premenopausal range at 6 months, while at 12 months, 3 patients had values above the upper premenopausal threshold and 3 above the postmenopausal range (Fig. 3).

No patient had values above the upper limit of the postmenopausal range for both P1NP and CTX after 12 months post-ZOL infusion.

Between groups, the percentage change of P1NP after 12 months in the ≤ 6 Group (77.3%) did not significantly differ ($P = 0.322$) from the relevant increase (100.4%) in the > 6 Group. This was also the case with the percentage increase in CTX in the ≤ 6 Group (72.2%) which did not differ ($P = 0.82$) from the increase in the > 6 Group (24%).

With the exception of a positive correlation ($r_s = 0.374$, $P = 0.01$) between P1NP values at 6 months

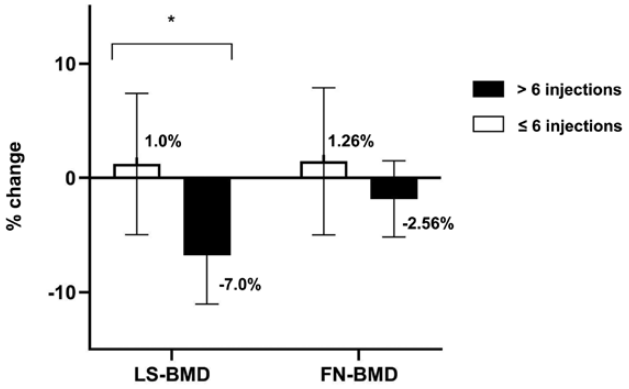

Figure 1. Percentage changes in bone mineral density after 12 months. Abbreviations: LS-BMD: bone mineral density lumbar spine, FN-BMD: bone mineral density femoral neck. *$P < 0.001$

Table 1. Baseline characteristics for treatment groups

Characteristics	≤ 6 injections (n = 27)	> 6 injections (n = 20)	*P* value
Age (years)	66.3 ± 9.13	64.80 ± 9.44	0.587
BMI (kg/m^2)	28.9 ± 3.85	24.00 ± 5.11	**<0.001**
BMD LS (g/cm^2)	0.98 ± 0.1	1.0 ± 0.11	0.6
BMD LS T-score	-1.70 ± 0.71	-1.5 ± 0.96	0.093
BMD FN (g/cm^2)	0.8 ± 0.08	0.78 ± 0.06	0.386
BMD FN T-score	-1.65 ± 0.65	-1.91 ± 0.54	0.351
P1NP (ng/mL)	25.10 ± 23.70	26.47 ± 29.36	0.858
CTX (ng/mL)	0.18 ± 0.12	0.25 ± 0.14	0.124
Vitamin D (nmol/L)	75.13 ± 30.90	92.67 ± 29.90	0.061
Calcium (mmol/L)	2.31 ± 0.11	2.36 ± 0.07	0.080
Phosphate (inorganic) (mmol/L)	1.15 ± 0.20	1.19 ± 0.21	0.520
Baseline fractures (nr of Fx)	0.48 ± 0.75	1.35 ± 1.5	**0.012**
Pts with Vertebral fractures	6	7	
Number of Dmab injections (median)	4	8	
Number of Dmab injections (mean)	4.4 ± 1.5	8.3 ± 1.2	**<0.001**
Years on Dmab treatment	2.2 ± 0.79	4.2 ± 0.6	**<0.001**

The time of zoledronate administration is considered as the "Baseline" time point. Years on Dmab treatment corresponds also with years with diagnosed osteoporosis as all patients were treatment-naïve before Dmab treatment.

Abbreviations: BMI, body mass index; BMD LS, bone mineral density of the lumbar spine; BMD FN, bone density measurement of the femoral neck; nr of Fx, number of fractures per patient; Pts, patients.

Table 2. Comparison (absolute values) of bone mineral density and bone turnover markers within and between groups

Variable	≤6 injections	>6 injections	Comparison between groups
			(*P* value)
BMD LS (g/cm^2)			
BL	0.98 ± 0.10	1.0 ± 0.11	0.6
12 months	0.99 ± 0.9	0.93 ± 0.12	0.052
Comparison within group	*P* = 0.409	***P* < 0.001**	
BMD FN (g/cm^2)			
BL	0.79 ± 0.09	0.78 ± 0.07	0.386
12 months	0.80 ± 0.07	0.76 ± 0.08	0.05
Comparison within group	*P* = 0.394	***P* = 0.034**	
P1NP (ng/mL)			
BL	25.10 ± 23.7	26.5 ± 29.4	0.858
6 months	30.7 ± 13.8	35.8 ± 12.7	0.196
12 months	44.5 ± 18.3[a,d]	53.1 ± 23.3[b,e]	0.232
Comparison within group	***P* = 0.001**[f]	***P* = 0.006**[f]	
CTx (ng/mL)			
BL	0.18 ± 0.15	0.25 ± 0.14	0.124
6 months	0.22 ± 0.11	0.25 ± 0.08	0.464
12 months	0.31 ± 0.16[c,d]	0.31 ± 0.14	0.966
Comparison within group	***P* < 0.001**	*P* = 0.275	

Abbreviations: BMD, bone mineral density; CTX, C-terminal telopeptide of type 1 collagen; FN, femoral neck; LS, lumbar spine; P1NP, procollagen type 1 N-terminal propeptide.
[a]*P* = 0.001 *vs* Baseline,
[b]*P* = 0.007 *vs* baseline,
[c]*P* < 0.001 *vs* baseline,
[d]*P* < 0.001 *vs* 6 months,
[e]*P* = 0.01 *vs* 6 months,
[f]Greenhouse-Geisser correction

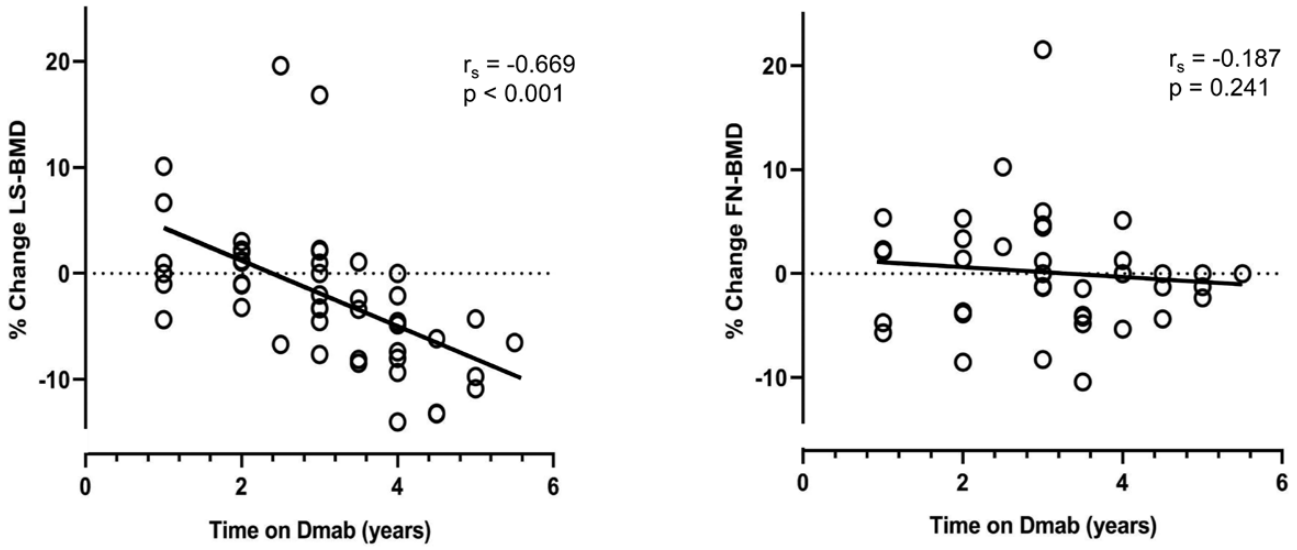

Figure 2. Correlation of time on Dmab treatment with changes in lumbar spine (LS) and femoral neck (FN) BMD.

and the years on Dmab treatment in the total cohort, no other correlations were found between bone turnover markers (BTMs) and the rest continuous variables of the study.

Fractures

During the study, 1 patient of the > 6 Group sustained a clinical vertebral fracture 12 months after ZOL. No other fractures were observed.

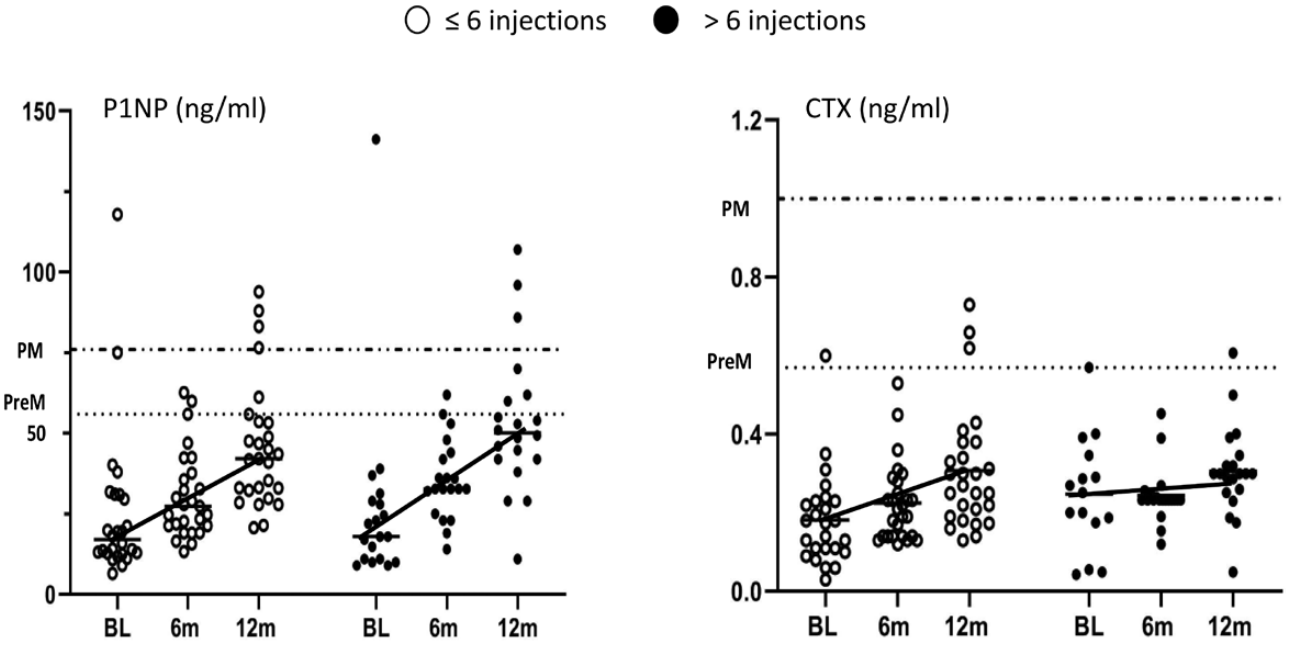

Figure 3. Distribution of bone turnover markers in both groups throughout the study. Solid lines = mean values, also joined for every group of patients. Abbreviations: PM, upper limit of postmenopausal range; P1NP 76 ng/mL, CTX 1.0 ng/mL; PreM, upper limit of premenopausal range; P1NP 56 ng/mL, CTX 0.57 ng/mL. BL, Baseline; m, months.

Adverse Events

Twenty-three (49%) of the 47 patients developed symptoms compatible with a transient acute phase reaction that was symptomatically treated with paracetamol. No other adverse events were recorded. No cases of osteonecrosis of the jaw or atypical femoral fracture were observed.

Discussion

In women with postmenopausal osteoporosis who became osteopenic with Dmab therapy for up to 3 years, a single ZOL 5 mg infusion given 6 months after the last Dmab injection, maintained the BMD gains at the spine and the hip for 1 year. In contrast, women treated for periods longer than 3 years with Dmab experienced significant BMD losses at both skeletal sites (7% and 2.6%, respectively). Duration of Dmab treatment is, therefore, an important determinant of the response to ZOL as further demonstrated by the significant negative correlation between length of Dmab treatment and changes in LS-BMD. Our results confirm and extend recent observations and recommendations by Sølling et al (7) and European Calcified Tissue Society (ECTS) experts (3), respectively, by providing evidence of the efficacy of ZOL over a broad time interval of Dmab use. Sølling et al reported that 20 patients with osteoporosis who were treated with Dmab for a mean of 5.2 years and received ZOL 6 months after the last Dmab injection lost 4.8% LS-BMD and 3.0% FN-BMD after 1 year (7), results very similar to ours. Furthermore, our data provide full support to the recommendation of ECTS experts for different management strategies of patients treated with Dmab for less than 2.5 years compared with those treated for longer periods (3). Notably, in the study of Sølling et al (7) 90% of the patients had received bisphosphonates before starting Dmab. It appears, therefore, that earlier bisphosphonate treatment does not affect the response, as also suggested in a larger observational study (8).

The obvious question is whether the difference in response to ZOL between the 2 groups in our study is related to differences in BTM changes following Dmab discontinuation. It has been previously suggested that the magnitude of the rebound phenomenon is related to the duration of Dmab treatment, possibly due to longer-term inhibition of osteoclast differentiation, which leads to a larger pool of osteoclast precursors that could differentiate synchronously upon Dmab withdrawal (8). It may, therefore, be that the ZOL 5 mg infusion may not be adequate to control a postulated greater stimulation of bone resorption caused by the longer duration of Dmab therapy. While this suggestion is pathophysiologically attractive, it is not supported by our data. Neither baseline nor follow-up levels of bone turnover markers differed between the 2 groups of patients. Although serum P1NP values at 6 months were positively correlated with treatment duration, in both groups of studied women significant changes in BTMs of similar magnitude occurred between 6 and 12 months after the ZOL infusion and in no patient in either group an increase in both serum P1NP and CTX values above the upper limit of the postmenopausal range was observed. Differences,

therefore, in bone turnover that may explain differences in BMD responses in our study are unlikely. The possibility of a transient, early difference in bone marker kinetics between the groups cannot be excluded but in published studies of BTM measurements after cessation of Dmab treatment, peak levels were observed between 6 and 12 months independently of ZOL use (1, 4). Furthermore, based on the findings of our study, the only one at present to include exclusively treatment-naïve patients, we could not identify a value of either serum CTX or P1NP that might help in the early identification of patients at risk for higher bone loss requiring adjustment of the management. The ECTS group recommended to monitor BTM at 3 and 6 months after ZOL and in case of increased BTMs above the mean of age- and sex-matched cohorts to consider a new infusion of ZOL. Although the recommendation is rational, our results cannot support this notion as no significant changes were observed in the 6-month BTMs.

Our 2 studied groups of women not only had different duration of exposure to denosumab but also differed significantly in 2 major independent risk factors of bone fragility, namely, BMI and number of prevalent fractures. Women with longer Dmab exposure had lower BMI values and higher number of prevalent fractures, suggesting that this group had more severe osteoporosis, this being the reason they received Dmab for longer periods to increase BMD to T-score values higher than –2.5. This speculation is also supported by the findings of the Sølling et al study (7). It may, therefore, be that the state of the disease is an important determinant of the response to Dmab treatment and its discontinuation. More severe disease requires longer treatment, which when stopped leads to greater BMD losses toward the original values. In our patients, the period since the recognition of the disease is clear because all patients were started on Dmab, whereas in most reports the majority of studied patients had already received bisphosphonates, the specific pharmacological properties of which may complicate the analysis of the responses. Whether there is an intrinsic mechanism that defines BMD levels at a given time for each untreated individual is currently unknown. However, if this is the case, and the skeleton of each individual tends to return to its pretreatment status, previously described as mechanostatic reset to a lower bone mass (13), it may explain the cause of a BTM-independent failure of ZOL to maintain the Dmab-induced BMD gains among patients with more severe disease. Unfortunately, BMD values before initiating Dmab therapy were, by design, not included in our study and we cannot, therefore, test this hypothesis, which warrants further investigation. An alternative, not mutually exclusive, mechanism may be related to the pharmacodynamic properties of Dmab in osteoporosis. In a bone biopsy study of osteoporotic women treated with Dmab for 10 years the degree of bone matrix mineralization increased in patients who received Dmab for 2 or 3 years vs placebo. With continuing treatment, matrix mineralization increased further significantly from years 2 or 3 to year 5 but not thereafter (14). Thus, during treatment with Dmab for 5 years more mineral was added to bone compared with 2 or 3 years for a similar reduction of bone remodeling (14). Accordingly, more mineral should have been added to bone in the > 6 Group of women in our study compared with that added to the bone of the women of the ≤ 6 Group. After discontinuation of Dmab, a ZOL 5 mg infusion—which induced similar changes in BTMs in the 2 groups—while sufficient to prevent the loss of the added mineral in the women of the ≤ 6 Group was insufficient to fully prevent the loss of the higher load of added mineral in the women of the > 6 Group. The result was maintenance of BMD in the former group and decrease in the latter. This hypothesis is compatible with the demonstrated relationship with the length of Dmab treatment as well as with the findings of all 3 prospective studies of the efficacy of bisphosphonates in patients discontinuing Dmab (4, 7, 15). Independently of the mechanism underlying the response, it is notable that in 91.5% of our patients BMD values remained osteopenic 1 year after ZOL administration. This finding in combination with the low rate (2.1%) of vertebral fractures justifies the selection of ZOL in a therapeutic strategy of patients with osteoporosis according to a "treat-to-target" approach targeting a total hip T-score between –1.5 and –2.0 (16).

The main limitation of our study is the lack of randomization due to the design of the analysis. Consequently, the 2 groups are not equal in size although they had been treated and followed prospectively according to the same protocol. However, the study allowed the systematic comparison of BMD and BTM changes among patients with a different duration of Dmab treatment who received ZOL 6 months following its discontinuation; the lack of early blood sampling may be considered an additional limitation.

In conclusion, the duration of Dmab treatment is a significant determinant of the overall BMD response after a single ZOL infusion among patients discontinuing Dmab treatment. A pragmatic approach would be to follow closely patients with longer than 3-year history of Dmab therapy as these may need additional therapeutic interventions in order to consolidate the BMD gains of previous treatment.

Acknowledgments

Financial Support: This work was not supported by any grant.

Author Contributions: Conception and design of the work: PM, NMA-D, SEP, ADA. Acquisition, analysis, or interpretation of data:

PM, NMA-D, SEP, SvW, EMW, SAP, MPY, ADA. Drafting the work: PM, NMA-D, ADA. Revising the work critically for important intellectual content: SEP, SAP. Final approval of the submitted version: PM, NMA-D, SEP, ADA, EMW, SvW, SAP, MPY. Agreeing to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: PM, NMA-D, SEP, ADA.

***Affirmation of Originality*:** All authors affirm that the work submitted for publication is original and has not been published other than as an abstract or preprint in any language or format and has not been submitted elsewhere for print or electronic publication consideration.

Additional Information

Correspondence: Athanasios Anastasilakis, MD, PhD, Ring Road, 564 29 N. Efkarpia, Thessaloniki, Greece. Email: a.anastasilakis@gmail.com.

Disclosures: PM reports fees for lectures/advisory boards and research grants from Amgen and fees for lectures/advisory boards from Glaxo, Lilly, UCB, Elpen, and Vianex; NMA-D has participated in the Dutch denosumab Advisory Board; SEP has received consulting/speaking fees from Amgen; EMW declares lecture honoraria from Amgen and Shire. SvW, SAP, and MPY have nothing to declare; ADA reports lecture fees from Amgen, Bianex, Eli-Lilly, ITF, Unifarma, and UCB

Data Availability: All datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Bone HG, Bolognese MA, Yuen CK, et al. Effects of denosumab treatment and discontinuation on bone mineral density and bone turnover markers in postmenopausal women with low bone mass. *J Clin Endocrinol Metab*. 2011;**96**(4):972-980.
2. McClung MR, Wagman RB, Miller PD, Wang A, Lewiecki EM. Observations following discontinuation of long-term denosumab therapy. *Osteoporos Int*. 2017;**28**(5):1723-1732.
3. Tsourdi E, Zillikens MC, Meier C, et al. Fracture risk and management of discontinuation of denosumab therapy: a systematic review and position statement by ECTS. *J Clin Endocrinol Metab*. 2020. doi: 10.1210/clinem/dgaa756.
4. Anastasilakis AD, Papapoulos SE, Polyzos SA, Appelman-Dijkstra NM, Makras P. Zoledronate for the prevention of bone loss in women discontinuing denosumab treatment. A prospective 2-year clinical trial. *J Bone Miner Res*. 2019;**34**(12):2220-2228.
5. Makras P, Papapoulos SE, Polyzos SA, Appelman-Dijkstra NM, Anastasilakis AD. The three-year effect of a single zoledronate infusion on bone mineral density and bone turnover markers following denosumab discontinuation in women with postmenopausal osteoporosis. *Bone*. 2020;**138**:115478.
6. Everts-Graber J, Reichenbach S, Ziswiler HR, Studer U, Lehmann T. A single infusion of zoledronate in postmenopausal women following denosumab discontinuation results in partial conservation of bone mass gains. *J Bone Miner Res*. 2020;**35**(7):1207-1215.
7. Sølling AS, Harsløf T, Langdahl B. Treatment with zoledronate subsequent to denosumab in osteoporosis: a randomized trial. *J Bone Miner Res*. 2020;**35**(10):1858-1870.
8. Everts-Graber J, Reichenbach S, Gahl B, Ziswiler HR, Studer U, Lehmann T. Risk factors for vertebral fractures and bone loss after denosumab discontinuation: a real-world observational study. *Bone*. 2021;**144**:115830.
9. Lehmann T, Aeberli D. Possible protective effect of switching from denosumab to zoledronic acid on vertebral fractures. *Osteoporos Int*. 2017;**28**(10):3067-3068.
10. Reid IR, Horne AM, Mihov B, Gamble GD. Bone loss after denosumab: only partial protection with zoledronate. *Calcif Tissue Int*. 2017;**101**(4):371-374.
11. Anastasilakis AD, Polyzos SA, Yavropoulou MP, et al. Comparative effect of zoledronate at 6 versus 18 months following denosumab discontinuation. *Calcif Tissue Int*. 2021;**108**(5):587-594.
12. Kondo H, Okimoto N, Yoshioka T, et al. Zoledronic acid sequential therapy could avoid disadvantages due to the discontinuation of less than 3-year denosumab treatment. *J Bone Miner Metab*. 2020;**38**(6):894-902.
13. Ferrari S. Zoledronate following denosumab discontinuation: partial reassurance but no confidence. *J Bone Miner Res*. 2020;**35**(7):1205-1206.
14. Dempster DW, Brown JP, Fahrleitner-Pammer A, et al. Effects of long-term denosumab on bone histomorphometry and mineralization in women with postmenopausal osteoporosis. *J Clin Endocrinol Metab*. 2018;**103**(7):2498-2509.
15. Freemantle N, Satram-Hoang S, Tang ET, et al; DAPS Investigators. Final results of the DAPS (Denosumab Adherence Preference Satisfaction) study: a 24-month, randomized, crossover comparison with alendronate in postmenopausal women. *Osteoporos Int*. 2012;**23**(1):317-326.
16. Ferrari S, Libanati C, Lin CJF, et al. Relationship between bone mineral density T-score and nonvertebral fracture risk over 10 years of denosumab treatment. *J Bone Miner Res*. 2019;**34**(6):1033-1040.