The Journal of Clinical Endocrinology & Metabolism, 2024, **00**, 1–10
https://doi.org/10.1210/clinem/dgae224
Advance access publication 13 April 2024
Clinical Research Article

Zoledronate After Denosumab Discontinuation: Is Repeated Administrations More Effective Than Single Infusion?

Giorgia Grassi,[1] Alberto Ghielmetti,[2] Marta Zampogna,[2] Iacopo Chiodini,[3,4] Maura Arosio,[1,2] Giovanna Mantovani,[1,2] and Cristina Eller-Vainicher[1]

[1]Endocrine Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[2]Department of Clinical Sciences and Community Health, University of Milan, 20100 Milan, Italy
[3]Department of Medical Biotechnology and Translational Medicine, University of Milan, 20100 Milan, Italy
[4]Unit of Endocrinology, ASST Grande Ospedale Metropolitano Niguarda, 20162 Milan, Italy

Correspondence: Giorgia Grassi, MD, Endocrine Unit, Fondazione IRCCS Ca" Granda-Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy. Email: dr.giorgiagrassi@gmail.com.

Abstract

Background: After denosumab (Dmab) discontinuation C-terminal telopeptide (CTX) levels increase, bone mineral density (BMD) decreases and multiple vertebral fractures (FX) may occur with relevant impacts on women's health. A sequential therapy with bisphosphonates is recommended, and the European Calcified Tissue Society (ECTS) proposed repeated zoledronate (ZOL) administrations in patients with persistently high CTX levels, although the efficacy of this schedule is unknown. In this retrospective study, we describe BMD changes and FX rate in 52 patients managed according to the ECTS recommendations.

Methods: We measured CTX levels and administered ZOL after 1 month from Dmab withdrawal (t0). After 6 months (t1), we administered a second ZOL infusion, if CTX levels were ≥280 ng/L. BMD changes and FX rate were assessed on average after 17 months from Dmab withdrawal.

Results: Seventy-five percent of patients repeated ZOL infusion. In this group, spine BMD declined significantly (−5.5 ± 5.6%), while it remained stable in the group with CTX levels <280 ng/L (−0.1 ± 5.5%, $P = 0.008$). All fractured patients (9.6%) had received >5 Dmab injections and 2 ZOL infusions. The BMD worsening after Dmab withdrawal was associated with CTX t1 [odds ratio (OR) 2.9, interquartile range (IQR) 1.3-6.6, $P = .009$] and spine BMD gain during Dmab therapy corrected for the number of Dmab injections (OR 3.0, IQR 1.2-7.2, $P = .014$). A CTX level at t1 > 212 ng/L had 100% sensitivity in predicting the BMD loss.

Conclusion: In patients with uncontrolled CTX levels after Dmab withdrawal, 2 ZOL infusions 6 months apart do not prevent BMD loss and FX.

Key Words: denosumab withdrawal, rebound effect, zoledronate, sequential therapy, osteoporosis, bone turnover markers

Denosumab (Dmab) is a monoclonal antibody against the receptor activator of nuclear factor κB ligand, which suppresses the differentiation from precursors cells and osteomorphs (end-stage nonresorbing cells derived from osteoclasts that can be recycled back into active osteoclasts) to osteoclasts. Dmab is administered subcutaneously at a dosage of 60 mg every 25 weeks to increase bone mineral density (BMD) and to reduce fracture (FX) risk in patients affected by osteoporosis or as primary prevention in patients taking glucocorticoids or hormonal deprivation therapy for prostate or breast cancer (1, 2).

Although a good safety profile is guaranteed for up to 10 years of treatment, Dmab may be withdrawn earlier in some cases, as in osteoporotic patients if the BMD improves and reaches osteopenic values or in patients without osteoporosis who discontinue hormonal deprivation therapy or glucocorticoids (1). It is known that, after Dmab withdrawal, bone turnover markers (BTMs) persistently increase, BMD rapidly falls to pretreatment values, and multiple clinical vertebral FX (VFX) may occur with relevant impacts on women's health (rebound phenomenon) (3-6). In particular, rebound VFXs are more likely if the Dmab therapy duration is longer than 3 years (7). By blocking the receptor activator of nuclear factor κB ligand, Dmab causes the osteoclasts precursors cells accumulation, so when Dmab is discontinuated, the osteoclastogenesis is enhanced. It has been suggested that the longer the duration of Dmab therapy (and the higher the number of the accumulated cells), the greater the extent of the "rebound," making the occurrence of VFX more probable (8).

Although the use of a sequential therapy with bisphosphonates (BPs) such as intravenous zoledronate (ZOL) can attenuate the rebound phenomenon, a single ZOL administration may not completely prevent the rebound VFX and BMD loss, in particular if more than 5 injections of Dmab have been administered (9-11).

In 2020 a position statement of the European Calcified Tissue Society (ECTS) recommended a sequential therapy with oral BPs or ZOL for 1 to 2 years depending on BTMs and BMD if the Dmab therapy duration was short (≤2.5 years), whereas in patients treated for longer periods (ie, >2.5 years) ZOL should be administered 6 months after the last injection and

Received: 18 January 2024. **Editorial Decision:** 1 April 2024. **Corrected and Typeset:** 17 April 2024

possibly repeated 3-6 months later in case of BMTs levels remaining still above the healthy premenopausal women threshold (C-terminal telopeptide, CTX ≥280 ng/L or procollagen type 1 N-terminal propeptide ≥35 µg/L) (12).

Notwithstanding these suggestions, so far no study is available proving that a repeated ZOL schedule could be more effective than a single infusion in patients with persistently elevated BTMs.

This retrospective real-life monocentric study was designed to evaluate the efficacy of a second ZOL administration in preventing BMD loss and VFX in a sample of consecutive patients who discontinued Dmab with persistent elevated CTX 6 months after the first ZOL infusion.

Patients and Methods

Patients

In this observational study, we retrospectively examined the available data in December 2023 of all patients referred to our outpatient clinic for metabolic bone diseases who discontinued Dmab therapy between January 2020 and June 2022 and underwent their first ZOL administration between 6 and 7 months after the last Dmab injection (n = 109). We excluded subjects (1) with secondary forms of osteoporosis (n = 9); (2) who lacked BMD measurement by dual-energy x-ray absorptiometry and/or of thoracolumbar spinal radiographs at the time of Dmab discontinuation (n = 1) or at follow-up (n = 44); (3) who refused to repeat ZOL in case of CTX ≥280 ng/L 6 months after the first administration (n = 4). Eventually, we enrolled 52 patients (3 males and 49 females). According to the Italian prescription rules, all patients had been treated for primary osteoporosis (n = 43) or for concomitant therapy with aromatase inhibitors (AI; n = 9). The decision for discontinuing Dmab was due to 1 of the following reasons: (1) presence of BMD at lumbar spine (LS) and total hip (TH) above −2.5 (n = 31); (2) AI discontinuation in the absence of other criteria to continue Dmab therapy (n = 9); (3) patient's will to discontinue Dmab (n = 10). All patients were taking adequate vitamin D and calcium supplements, if needed.

Ethical approval was obtained from the ethic committee (Milan, Lombardia 3) (ID: 3648, response November 20, 2023).

Methods

From all enrolled patients, data on body mass index, smoking habits, family history of osteoporosis and hip fractures, and personal history of FX were collected. At the time of Dmab discontinuation (t0), FXs were considered prevalent and due to bone fragility if they occurred before or during Dmab therapy and without any evident trauma or after a low-energy trauma (eg, a fall from a standing height).

In all patients CTX, osteocalcin, calcium, creatinine, total, and bone-specific alkaline phosphatase (bALP) were measured on the same day of the first ZOL administration (t0) and 6 months later (t1) in the early morning at the central clinical routine laboratory of our hospital. The normal upper limit of CTX in our laboratory is <584 ng/L in premenopausal women, <1008 ng/L in postmenopausal women, and <854 ng/L in men. According to the ECTS position statement, we administered an additional ZOL dose 6 months after the first 1 in patients with CTX at t1 ≥ 280 ng/L.

The BMD was measured by dual-energy x-ray absorptiometry (Hologic Discovery, software version 13.3:3, Bedford, MA) at the LS (L1–L4) (Z-LS, in vivo precision 1.0%), femoral neck (FN, Z-FN, in vivo precision 1.8%), and TH (Z-TH, in vivo precision 1.7%) at the time of Dmab discontinuation and at follow-up. We calculated the LS, FN, and TH-BMD changes after Dmab discontinuation. When available, we also calculated the BMD changes during Dmab therapy and the overall BMD changes from the Dmab initiation until the end of the study follow-up. The BMD variation was considered significant if above or below the least significant change (LSC) (LS: 2.8%, FN: 5.9%, TH: 4.8%). We defined a BMD worsening in case of a loss > LSC at ≥2 sites measured (LS, FN, and TH) or in case of isolated LS BMD loss >5%, regardless of FN and/or FT trend. We decided to consider even this latter condition on the basis of other authors' experience in earlier studies on this topic (13, 14). The BMD measurement during follow-up was performed 17 ± 3 months after Dmab discontinuation.

Information on incident clinical fragility FX after Dmab discontinuation was obtained from all subjects at the regularly scheduled clinical visits, and they were confirmed by reviewing medical records. We also encouraged patients to contact us in case of ascertained FX or symptoms suggesting FX.

A conventional spinal radiograph in lateral and anteroposterior projection (T4–L4) was obtained in all subjects at the time of Dmab discontinuation and at follow-up using a standardized technique. Morphometric vertebral FX was diagnosed using the semiquantitative visual assessment (SQ) (15). FXs were defined as reductions of >20% in anterior, middle, or posterior vertebral height. From lateral spine radiographs, 13 vertebrae from T4 to L4 were assessed visually as intact (SQ grade 0) or as having approximately mild (20% to 25% height reduction), moderate (25% to 40% height reduction), or severe (>40% height reduction) deformity (SQ grades 1, 2, and 3, respectively) (15). In all patients, we calculated the spinal deformity index, which is considered as a tool for assessing future VFX risk (16), by summing the fracture grades of all vertebrae (T4 to L4). Two radiologists, who were blinded to BMD data, independently reviewed the radiographs.

All the information given by patients was confirmed by reviewing the medical records. Traumatic FXs were not considered in the analysis.

Statistical Analysis

Statistical analysis was performed by SPSS version 28.0 statistical package (IBM). The results were expressed as mean ± SD or median (IQR). The normality of distribution was tested by Kolmogorov–Smirnov test. The comparison of continuous variables was performed using Student's *t*-test or Mann–Whitney U-test, as appropriate. Categorical variables were compared by χ^2 test or Fisher's exact test, as appropriate. We performed the following comparisons: (1) characteristics of patients with indication to second ZOL administration with those of patients without indication to second ZOL administration; (2) characteristics of patients who maintained BMD with those of patients who lost BMD after Dmab withdrawal; (3) characteristics of patients with short, medium, and long duration of Dmab therapy. The linear regression analysis assessed the association between the percentage change in LS BMD (ΔLS) after Dmab and the number of Dmab injections, the CTX levels at t0 and t1. The logistic regression analysis

Table 1. The clinical, biochemical, and radiological findings of all patients discontinuing denosumab and the comparison between patients who underwent ZOL1 or ZOL2

	All patients (52)	ZOL1 (13)	ZOL2 (39)	*P*
Age (years)	71 ± 8.5	73.1 ± 7.0	70.0 ± 8.9	.263
BMI (kg/m^2)	24.5 ± 4.8	25.5 ± 5	24.1 ± 4.4	.369
Sex (male)	3 (5.8)	2 (15.4)	1 (2.6)	.086
No. of Dmab injections	8 ± 3	8 ± 3	9 ± 3	.222
Dmab for ≥2.5 years	43 (82.7)	10 (76.9)	33 (84.6)	.525
Aromatase inhibitors therapy	9 (17.3)	1 (7.7)	8 (20.5)	.290
Spine Deformity Index	1 (3)	2 (3)	1 (4)	.773
Previous fractures	34 (65.4)	8 (61.5)	26 (66.7)	.747
Previous BPs therapy	23 (44.2)	6 (46.2)	17 (43.6)	1.000
TBS	1.128 (0.128)	1.129 (0.192)	1.134 (0.128)	.429
ΔLS% in Dmab	14.8 ± 8.5	11.5 ± 12.0	15.9 ± 6.9	.137
ΔFN% in Dmab	4.9 ± 5.1	4.9 ± 7.8	4.9 ± 4.3	.922
ΔTH% in Dmab	6.8 ± 5.4	5.1 ± 5.3	7.3 ± 5.4	.276
LS T-score t0	−2.4 ± 0.9	−2.5 ± 1.1	−2.3 ± 0.8	.517
FN T-score t0	−2.2 ± 0.8	−2.2 ± 1.0	−2.1 ± 0.8	.777
TH T-score t0	−1.7 ± 0.9	−1.8 ± 1.1	−1.7 ± 0.8	.722
ΔLS% after Dmab	−4.4 ± 6.0	−0.1 ± 5.5	−5.5 ± 5.6	.008
ΔFN% after Dmab	−2.1 ± 6.3	1.2 ± 7.5	−3.0 ± 2.7	.073
ΔTH% after Dmab	−1.7 ± 4.3	0.4 ± 6.3	−2.1 ± 3.7	.323
CTX t0 (ng/L)	415 ± 343	342 ± 227	442 ± 375	.363
ALP t0 (U/L)	57 (24)	57 (25)	57 (24)	.960
bALP t0 (μg/L)	8.4 ± 3.7	7.3 ± 3.3	8.7 ± 3.8	.234
OC t0 (μg/L)	13.7 ± 4.5	12.8 ± 5.3	14.0 ± 4.3	.467

Data are expressed as mean ± SD, median and interquartile range, or absolute value and percentage in parentheses.
Abbreviations: ALP, total alkaline phospatase; bALP, bone-specific alkaline phospatase; BMD, bone mineral density; BMI, body mass index; BPs, bisphosphonates; CTX, C-telopeptide of type 1 collagen; Dmab, denosumab; FN, femoral neck; LS, lumbar spine; OC, osteocalcin; TBS, trabecular bone score; TH, total hip; ZOL1, patients who underwent a single ZOL infusion; ZOL2, patients treated with an additional ZOL administration 6 months later; ZOL, zoledronate.

assessed the association between the LS BMD worsening after Dmab discontinuation, the CTX levels at t1, the LS BMD gain during Dmab therapy, and the number of Dmab injections. Modifications of BMD have been expressed as percentage (Δ) changes vs Dmab withdrawal or vs Dmab initiation, as specified.

The receiver operating characteristic curve was used for assessing the cut-off of CTX value with the best diagnostic accuracy for predicting the BMD worsening after Dmab discontinuation. *P*-values of less than .05 were considered significant.

Results

The clinical, biochemical, and radiological parameters of all patients (n = 52) at the time of Dmab discontinuation (t0) are summarized in Table 1.

Mean BMD changes during Dmab therapy and after discontinuation are summarized in Fig. 1. All patients showed a BMD improvement at all sites during Dmab. As compared with BMD at the Dmab initiation, however, at the end of the follow-up the BMD increase obtained with Dmab therapy was significantly maintained only at LS. After Dmab discontinuation, the percentage of subjects with BMD loss ≥ LSC was 61% at LS, 38% at FN, and 23% at TH.

Overall, 5 female patients (9.6%) presented a FX during the follow-up after Dmab discontinuation. Only 1 of them presented multiple clinical VFX, which have been typically associated with the "rebound," that occurred 6.5 months after the last Dmab injection and before the first ZOL administration. The clinical, radiological, and biochemical findings of patients with incident FX after Dmab withdrawal are detailed in Table 2. The prevalence of subjects with values < −2.0 was 22.6% (12 patients) in our cohort, and we found no difference in TH T-score at Dmab withdrawal between patients with or without rebound FX (−2.0 ± 1.2 vs −1.7 ± 0.9, $P = .517$).

We found that a comparable percentage of patients treated for AI or osteoporosis had indication to a second ZOL, and the percentages of patients with the BMD worsening at follow-up were also comparable in the 2 groups (data not shown). Among patients with incident FX, none had been treated with Dmab for AI therapy.

Comparison Between Patients With and Without Indication to Repeat a Second Dose of ZOL After 6 Months

The comparison between patients who underwent a single ZOL infusion (ZOL1; n = 13) or an additional ZOL administration 6 months later (ZOL2; n = 39) is reported in Table 1. All parameters at t0 considered in the analyses were comparable between the 2 groups. In particular, the number of Dmab injections and the number of subjects treated with Dmab for ≥2.5 years was comparable between ZOL1 and ZOL2 patients.

The percentage of subject with LS, FN, and TH loss > LSC after Dmab discontinuation was not significantly different between the 2 groups (5 vs 24%, $P = .473$; 3 vs 13%, $P = 1.000$; 0 vs 10%, $P = .168$; respectively, at LS, FN, and TH in ZOL1 and ZOL2). The mean BMD changes during Dmab therapy and after discontinuation in ZOL1 and ZOL2 patients are reported in Fig. 1. Both groups improved significantly at all sites during Dmab administration with no significant differences. After Dmab discontinuation in ZOL1 patients BMD remained stable while ZOL2 presented a BMD loss at LS and at femur, even though without reaching statistical significance for the latter site. Although the overall BMD variations between Dmab initiation and the end of the follow-up did not differ significantly between the 2 groups, ZOL1 patients showed a BMD improvement beyond the LSC at all sites while ZOL2 only at LS.

All patients with incident FX after Dmab discontinuation belonged to the ZOL2 group, with the rate of FX incidence being 12.8% in this group. No significant difference was found between patients with incident FX as compared to those without incident FX within the ZOL2 group in terms of body mass index, sex, age, or prevalent FX pre-Dmab; use of BPs pre-Dmab; number of Dmab injections and of patients treated with Dmab therapy longer than 2.5 years; ΔBMD at LS, FN, and TH during Dmab therapy, after Dmab discontinuation, and overall; prevalence of patients experiencing a BMD

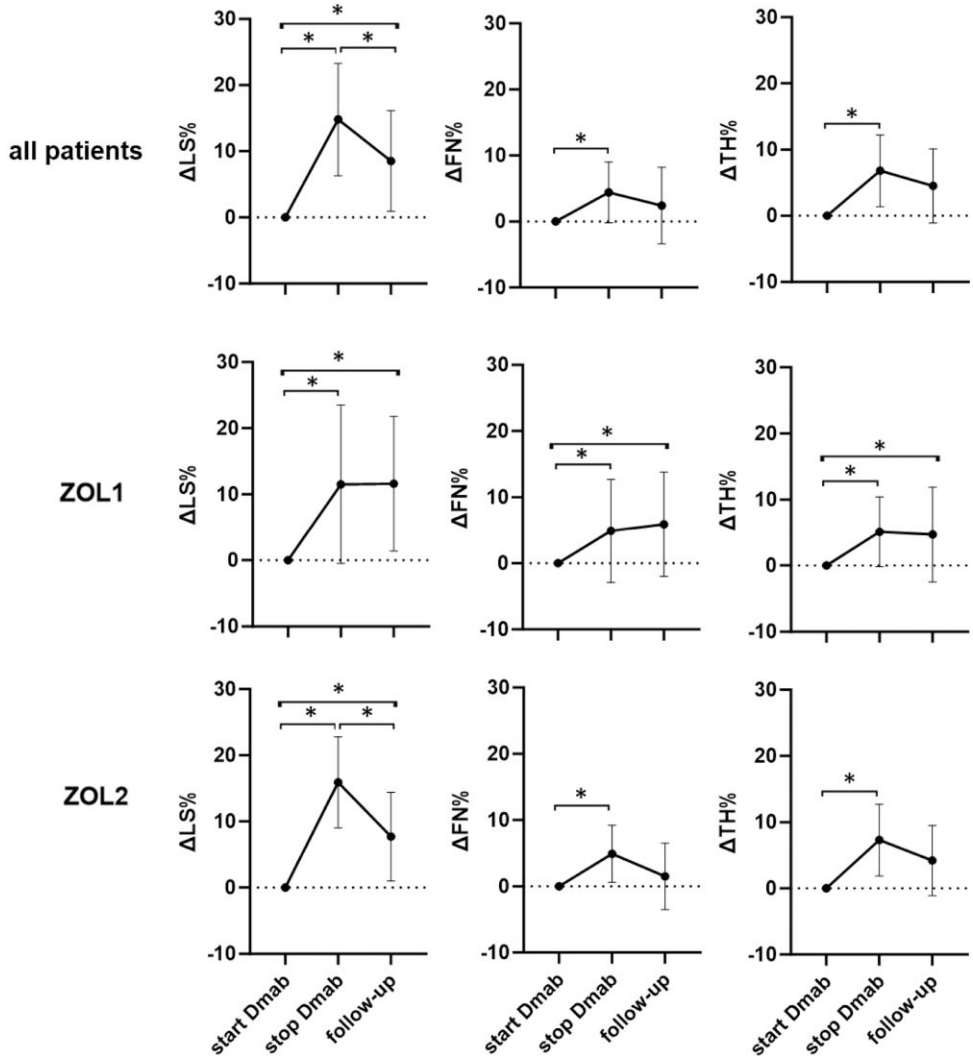

Figure 1. The BMD changes during Dmab therapy and after discontinuation in all patients and in the group treated with ZOL1 or in patients who underwent a second zoledronate dose 6 months later. Percent changes (mean ± SD) during Dmab treatment and after Dmab discontinuation in LS, FN, TH. *ΔBMD > LSC.

Abbreviations: BMD, bone mineral density; Dmab, denosumab; FN, femoral neck; LS, lumbar spine; LSC, least significant change; TH, total hip; ZOL, zoledronate; ZOL1, single zoledronate infusion; ZOL2, 2 zoledronate infusions after denosumab.

worsening after Dmab discontinuation and overall; or BTM levels at t0 and t1 (data not shown).

Comparison Between Patients With BMD Stability or Worsening at the End of Follow-up After Dmab Discontinuation

Among the group of patients defined as having a BMD worsening after Dmab discontinuation, 8 patients (29.6%) had an isolated LS BMD loss >5% (mean −8.8%, ranging from −6.3% to −12%) and 19 patients (70.4%) had a BMD loss ≥ LSC at ≥2 sites (2 subjects at FN + TH, 3 at LS + TH, 9 at LS + FN, and 5 at LS + FN + TH).

The comparison between patients who presented a BMD worsening (n = 25) or BMD stability (n = 27) at follow-up after Dmab discontinuation is reported in Table 3. The patients with BMD worsening after Dmab discontinuation presented a significantly higher BMD gain during Dmab therapy at LS and FN even after adjusting for the number of Dmab injections. Among patients who experienced a BMD worsening at follow-up, the prevalence of subjects with long Dmab therapy duration (≥2.5 years) was higher and the CTX levels at t0 tended to be higher as compared with patients who did not present the BMD worsening. At t1 the mean levels of CTX, alkaline phosphatase, and bone-specific alkaline phosphatase and the proportion of subjects with CTX ≥280 ng/L were found to be significantly higher in patients with BMD worsening at follow-up as compared with patients with BMD stability (Fig. 2).

The logistic regression analysis showed that the BMD worsening after Dmab discontinuation was significantly associated with both CTX at t1 and LS BMD gain in Dmab corrected for the number of Dmab injections regardless of the number of Dmab injections (Table 4).

Duration of Dmab Therapy and BMD Variation After Dmab Discontinuation

The LS BMD changes after Dmab discontinuation were associated with the number of Dmab injections ($R^2 = 0.210$,

Table 2. The clinical, radiological, and biochemical data of patients suffering fractures after Dmab discontinuation

No.	Sex	Age	AI	Prevalent FX before Dmab	No. of Dmab injections	FX in Dmab	Reason for Dmab withdrawal	No. of ZOL	Type of FX post-Dmab	Time of FX (months of Dmab washout)	TBS	LS T-score t0	FN T-score t0	TH T-score t0	ΔLS% post Dmab	ΔFN% post Dmab	ΔFT% post Dmab	CTX t0 (ng/L)	CTX t1 (ng/L)
1	F	59	No	Yes, cVFX	8	No	Patient's will	2	Multiple cVFX	0.5 (immediately before ZOL1)	1.055	−3.0	−2.9	−2.7	−3.0	−5.3	−0.7	1220	289
2	F	77	No	Yes, morpho VFX	8	No	T-score ≥ −2.5 at all sites	2	Single cVFX	6 (immediately before ZOL2)	1.137	−1.3	−0.9	0.1	−10.0	2.0	−4.0	1136	413
3	F	75	No	Yes, morpho VFX	10	Yes, single cVFX	T-score ≥ −2.5 at all sites	2	Single cVFX	17 (11 months after ZOL2)	1.040	−2.0	−2.0	−2.1	−5.1	−8.9	−2.9	537	295
4	F	74	No	Yes, cVFX and morpho VFX	6	No	Patient's will	2	MorphoVFX	? (x-ray performed at 18 months follow-up)	1.136	−3.4	−3.0	−2.3	−2.6	0.0	0.0	285	372
5	F	81	No	Yes, cVFX	6	No	Patient's will	2	Hip FX	18 (12 months after ZOL 2)	1.100	−2.5	−4.1	−2.9	−0.1	4.5	−4.4	176	384

Abbreviations: AI, aromatase inhibitor; CTX, C-terminal telopeptide; cVFX, clinical vertebral FX; Dmab, denosumab; F, female; FN, femoral neck; FX, fracture; LS, lumbar spine; morphoVFX, morphometric vertebral FX; TBS, trabecular bone score; TH, total hip; ZOL, zoledronate; ZOL1, single ZOL infusion; ZOL2, an additional ZOL administration 6 months later.

Table 3. Clinical, biochemical and radiological parameters at the time of Dmab discontinuation (t0) and 6 months later (t1) in patients who retained the BMD gained in Dmab or who presented BMD worsening after Dmab discontinuation

	BMD stable at follow-up (25)	BMD worsened at follow-up (27)	*P*
Age (years)	70.9 ± 8.9	70.0 ± 8.1	.725
BMI (kg/m2)	24.0 ± 5.1	24.8 ± 3.8	.539
Sex (male)	3 (12)	0 (0)	.166
No. of Dmab injections	8 ± 3	9 ± 3	.098
Dmab for ≥2.5 years	18 (72.0)	27 (95.7)	.050
Aromatase inhibitors therapy	5 (20.0)	4 (14.8)	1.000
Spine Deformity Index	2 (4)	2 (5)	.652
Previous fractures	17 (68.0)	20 (69.6)	1.000
Previous BPs therapy	10 (40.0)	15 (54.5)	.563
TBS t0	1.130 ± 0.071	1.135 ± 0.124	.906
LS T-score t0	−2.4 ± 0.9	−2.4 ± 0.8	.906
FN T-score t0	−2.3 ± 0.8	−2.1 ± 0.7	.365
TH T-score t0	−1.8 ± 0.8	−1.8 ± 0.8	.961
ΔLS in Dmab (%)	11.3 ± 8.0	18.8 ± 7.3	.003
ΔFN in Dmab (%)	2.3 ± 3.5	6.6 ± 5.0	.004
ΔTH in Dmab (%)	5.7 ± 5.5	8.2 ± 5.4	.162
ΔLS in Dmab/no. of injections (%)	1.6 ± 0.9	2.2 ± 1.1	.050
ΔFN in Dmab/no. of injections (%)	0.3 ± 0.5	0.8 ± 0.7	.034
ΔTH in Dmab/no. of injections (%)	0.8 ± 0.9	0.9 ± 0.6	.725
CTX t0 (ng/L)	335 ± 304	602 ± 450	.067
ALP t0 (U/L)	56 (25)	67 (24)	.182
bALP t0(μg/L)	8.1 ± 3.8	8.9 ± 3.7	.479
OC t0 (μg/L)	14 ± 5	14 ± 5	.891
CTX t1 (ng/L)	412 ± 253	588 ± 200	.011
CTX t1 > 280 ng/L	16 (62.2)	26 (91.3)	.036
ALP t1(U/L)	62 ± 13	74 ± 20	.035
bALP t1(μg/L)	10.8 ± 4.6	14.2 ± 5.2	.034
OC t1 (μg/L)	17.7 ± 5.6	21.4 ± 6.4	.130

Data are expressed as mean ± SD, median and interquartile range, or absolute value and percentage in parentheses.
Abbreviations: ALP, total alkaline phosphatase; bALP, bone-specific alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; BPs, bisphosphonates; CTX, C-terminal telopeptide of type 1 collagen; Dmab, denosumab; FN, femoral neck; LS, lumbar spine; OC, osteocalcin; TBS, trabecular bone score; TH, total hip; ZOL, zoledronate.

$P = .001$) (Fig. 3). We arbitrarily defined the duration of Dmab therapy as "short" if ≤5 injections were administered, "medium" if 6 to 13 injections were administered, and "long" if more than 14 injections were administered. The BMD changes in patients treated with Dmab for short (n = 9), medium (n = 29), or long (n = 14) time are reported in Fig. 2. We did not find any significant difference between the 3 groups in terms of overall BMD changes (between the start of Dmab and the end of the follow-up). Nevertheless, the percentage BMD variations at all sites after Dmab discontinuation were significantly different in the group treated for a

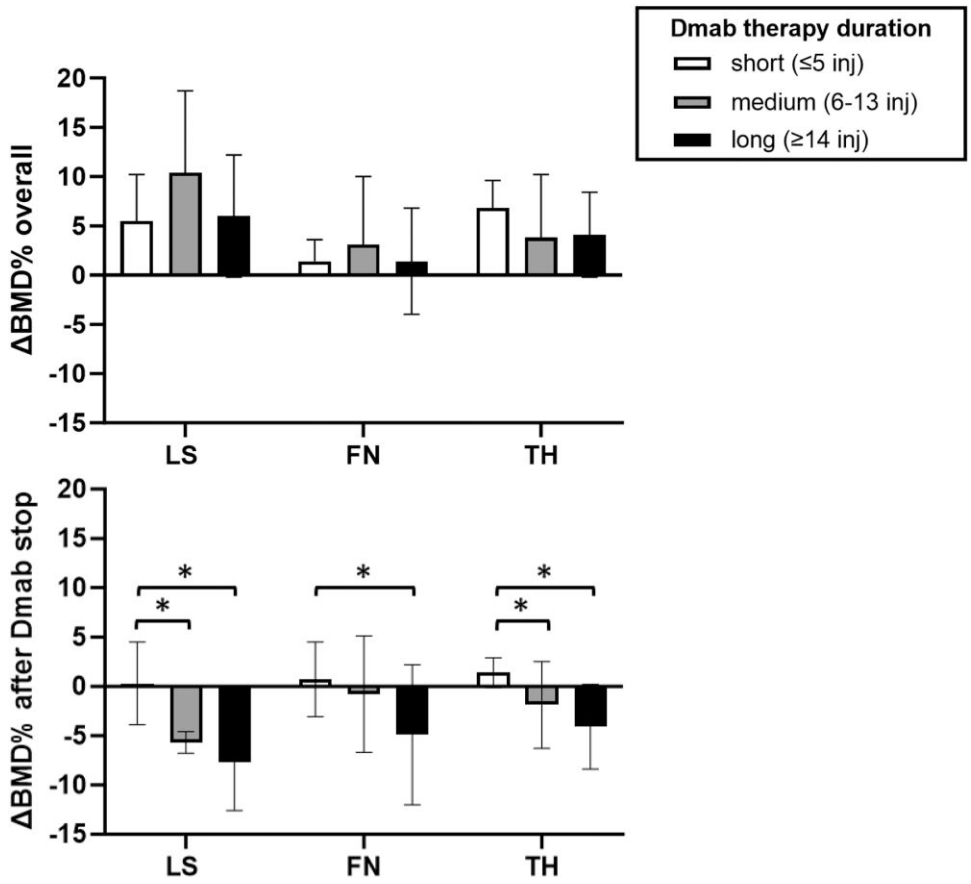

Figure 2. The BMD changes overall (since the start of Dmab till the end of the follow-up) and after Dmab discontinuation in patients with different Dmab therapy duration. Data are expressed as mean ± SD. *ΔBMD > LSC.

Abbreviations: BMD, bone mineral density; Dmab, denosumab; FN, femoral neck; Inj, injections; LS, lumbar spine; LSC, least significant change; TH, total hip.

Table 4. Odds ratio for bone mineral density worsening after Dmab discontinuation for potential risk factors using multivariate logistic regression analysis

	Odds ratio	95% CI	*P*
Dmab injections (for 1 increase)	1.3	0.9-1.8	.157
CTX t1 (for 1 quartile)	2.9	1.3-6.6	.009
ΔLS% in Dmab/n° Dmab injections	3.0	1.2-7.2	.014

Abbreviations: CI, confidence interval; CTX, C-telopeptide of type 1 collagen; Dmab, denosumab; LS, lumbar spine; ZOL, zoledronate.

short period of time as compared with both medium (0.3 ± 3.8 vs -4.7 ± 6.3, $P = .013$; 1.2 ± 1.5 vs -1.7 ± 4.4, $P = .034$; respectively at LS and TH) and long duration (0.3 ± 3.8 vs -6.8 ± 4.9, $P = .001$; 1.2 ± 1.5 vs -3.7 ± 4.2, $P = .050$; 0.7 ± 3.8 -5.1 ± 6.8, $P = .002$; respectively, at LS, TH, and FN).

Relation Between CTX Levels and BMD Variations After Dmab

The linear regression analysis showed that LS BMD changes after Dmab discontinuation were correlated with CTX levels both at t0 ($R^2 = 0.134$, $P = .016$) and at t1 (R^2 0.148, $P = .009$) (Fig. 4).

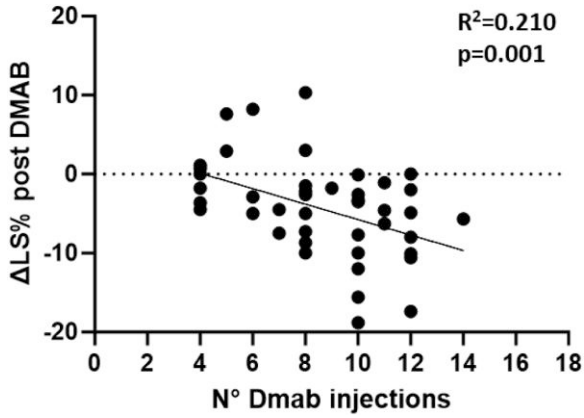

Figure 3. Correlation between the LS BMD changes after Dmab withdrawal and the number of Dmab injections.

Abbreviations: BMD, bone mineral density; Dmab, denosumab; LS, lumbar spine.

The receiver operating characteristic analysis showed that the CTX levels cut-off with the best compromise between sensitivity and specificity in order to discriminate patients with stable or worsened BMD was 453 ng/L (sensitivity 78%, specificity 67%) (Fig. 5). Nevertheless, CTX levels t1 > 212 ng/L had 100% sensitivity for identifying patients with worsened BMD at follow-up and might therefore be the cut-off to aim during the follow-up to obtain BMD stability.

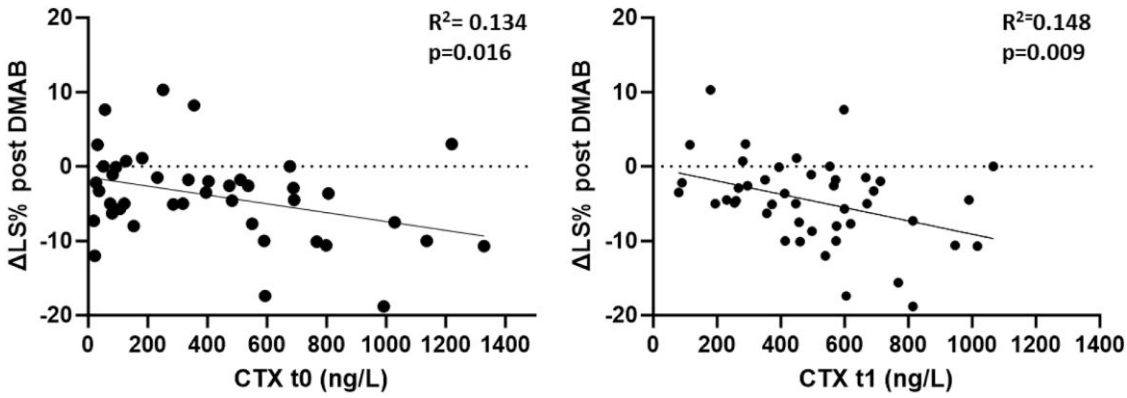

Figure 4. Correlation between the LS BMD changes after Dmab withdrawal and the CTX measured the day of the first ZOL (t0) or 6 months later (t1).
Abbreviations: BMD, bone mineral density; CTX, C-telopeptide of type 1 collagen; Dmab, denosumab; LS, lumbar spine; ZOL, zoledronate.

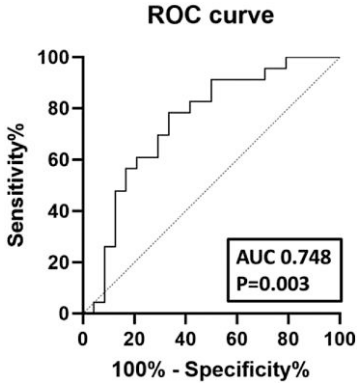

Figure 5. The ROC curve analysis of CTX 6 months after the first ZOL in discriminating patients with worsened bone mass density at follow-up after Dmab discontinuation.
Abbreviations: CTX, C-telopeptide of type 1 collagen; Dmab, denosumab; ROC, receiver operating characteristic; ZOL, zoledronate.

Discussion

This retrospective study explored the effects of a second ZOL administration in preventing BMD loss and VFX in a sample of consecutive patients who discontinued Dmab and had persistent elevated CTX at 6 months after the first ZOL infusion. In the present study, the majority of patients discontinuing Dmab had an indication to a second ZOL infusion at 6 months after the first 1, according to the ECTS recommendations (12). In spite of a second ZOL dose, these patients experienced a significant BMD loss at LS during the follow-up with a 12.8% FX incidence. On the other hand, patients with controlled CTX at 6 months after the first ZOL infusion had a BMD stability after Dmab withdrawal and, at the end of the follow-up, presented a significant BMD gain at all sites as compared with the time of Dmab initiation. The BMD changes after Dmab withdrawal were negatively correlated with the number of Dmab injections and with the CTX levels measured both on the day of the first ZOL and 6 months later. We found that a CTX >212 ng/L at 6 months after the first ZOL injection had 100% sensitivity in predicting the BMD loss. Finally, the LS BMD worsening after Dmab withdrawal was associated with the CTX levels at 6 months and with the degree of LS BMD gain during Dmab therapy regardless of the number of Dmab injections.

Although it is well known that Dmab discontinuation is followed by the loss of the BMD gained and possible FX occurrence, it is still not completely clear how to prevent this circumstance. The DATA-switch study highlighted how a subsequent therapy with teriparatide does not offer any advantage, whereas romosozumab might further increase the BMD (17, 18). Nevertheless, so far, most of the studies have focused on the use of a sequential therapy with BPs. In case of Dmab therapy duration <2.5 years, a sequential therapy with both alendronate and ZOL has been suggested to be sufficient to prevent the BMD loss and to reduce the occurrence of rebound FX after Dmab discontinuation. However, such therapies might not be effective if administered at the standard doses (ie, the doses approved to treat osteoporosis) if Dmab has been administered for longer time (5, 13, 19-24). Furthermore, the best timing of ZOL administration is still unknown, as the only randomized clinical trial (ZOLARMAB), which compared 3 different schedules (at 6 months, at 9 months, or on the basis of the CTX levels above the postmenopausal reference range) found a significant decrease in the BMD in all groups after 12 months, with no differences among the schedules (14, 23, 24).

In keeping with the available data, in 2020 the ECTS released a position statement about the management of Dmab discontinuation, where the authors recommended to always introduce a sequential therapy with BPs after Dmab, starting from 6 months after the last injection (12). Whereas patients treated for Dmab for <2.5 years can be either managed with oral BPs or intravenous ZOL, those treated for a longer time must undergo ZOL and subsequently CTX should be monitored 3 and 6 months later (10). The ECTS based the definition of an adequate response on the presence of CTX levels <280 ng/L (mean levels found in healthy premenopausal women), since previous data from the ReoLaus cohort showed that patients who retained BMD 12 months after discontinuing Dmab, during follow-up, had CTX close to this threshold (336 ng/mL) and suggested that in case of finding of higher levels, additional ZOL doses should be given (12, 25, 26).

Notwithstanding the ECTS position statement, very scarce data are available about the role of the CTX in predicting BMD loss and addressing the sequential antiresorptive treatment (in particular concerning the CTX cut-offs to be adopted for ZOL retreatment), and no data are available supporting the possible advantage of repeated ZOL

administrations in patients with not-controlled CTX levels. In the ZOLARMAB study, the authors established administration of an additional ZOL in case of CTX ≥1.26 ng/L (monitored every 6 months), but the cut-off used for CTX was very high and no patient fulfilled such criteria for retreatment (14). In a subsequent study (ProOff study), ZOL was administered 6 months after the last Dmab injection in all patients. The individuals treated with Dmab for longer than 2.5 years were subsequently monitored with CTX measurement every 3 months, and a second dose was given 6 months apart if CTX levels increased >2 fold during the follow-up (9). Twenty-four patients (31.6%) had to repeat ZOL after 6 months, but the BMD decreased at all sites with no differences between the group treated with double or single ZOL (9).

In this study, we report for the first time in a sample of consecutive patients who discontinued Dmab the effect of the systematic application of the ECTS suggestion to give a second ZOL in those with CTX ≥ 280 ng/L after the first ZOL infusion (12). All patients were treated with ZOL within 1 month of Dmab washout, and CTX levels were measured 6 months later, with ZOL being repeated in subjects with high levels. We pointed out that the majority of patients (75%) had an indication to repeat ZOL. No difference was found in terms of Dmab therapy duration between patients with or without indication to a second ZOL dose, suggesting that also patients with short Dmab therapy should be monitored to assess the need of a second ZOL dose. As a consequence, in case of unavailability of CTX, after 6 months a second ZOL might be considered as a preventive therapy, regardless of Dmab therapy duration.

From a clinical point of view, a further important finding of the present study is that patients who experienced a significant BMD loss during the follow-up (about 50%) where those with a more significant BMD improvement during Dmab therapy and that almost all these patients had the indication to a second ZOL infusion. Of note, the CTX levels measured on the day of the first ZOL and 6 months later were predictive of the LS BMD variation after Dmab discontinuation, as already described in earlier studies (14, 24). These findings shed light on the possibility of a precocious identification of patients who are at higher risk of BMD worsening after Dmab withdrawal. On the other hand, if controlled CTX levels are found after the first ZOL administration, physicians could be reassured about the possibility of retaining the BMD gained. In our study, the group of patients with BMD stable at follow-up had mean CTX levels at 6 months similar to those described in the ReoLaus study (412 vs 336 ng/L) (25). However, the best cut-off of CTX levels at 6 months for reliably identifying subjects with possible BMD loss after Dmab was as low as 212 ng/L, which is lower than the average levels observed in healthy women before menopause.

Although on the basis of the present data, patients with a short Dmab therapy should also be monitored to assess the need of a second ZOL dose, we found that the LS BMD variations after Dmab discontinuation were negatively correlated to the number of Dmab injections as highlighted even by previous studies (14, 24). In particular, in our study, subjects treated with Dmab for a short time (≤2.5 years) maintained the BMD at the follow-up, whereas subjects treated for a longer time tended to worsen with no difference between medium (3-6.5 years) and long (≥7 years) Dmab therapy duration, as also shown by another study by Everts-Graber and coauthors (9). Furthermore, although the overall BMD variations between Dmab initiation and the end of the follow-up after Dmab withdrawal were comparable in the 3 groups, all the incident FX occurred in patients belonging to the medium Dmab duration therapy. This finding, though to be confirmed in larger series, could be of interest from a clinical point of view. Indeed, in a recent review, a treat-to-target approach with Dmab was proposed, since in the FREEDOM trial the post hoc analyses showed a decreasing risk of rebound FX in patients with high TH T-score values up to −1.5 at the time of therapy discontinuation (27, 28). On the other hand, a treat-to-target approach could expose patients with very low BMD to many years of therapy, therefore potentially increasing the risk of rebound FX, since the multiple VFX incidence is known to be higher in patients treated for >3 years (7). Furthermore, such an approach does not take into account the bone microarchitecture, which improves during Dmab, as shown in the FREEDOM study through the trabecular bone score evaluation, but less impressively than BMD, and, as a result, it may be still deteriorated at the time of Dmab discontinuation (29). In general, we are aware that a treat-to-target approach with Dmab could be advised in patients at high risk of FX and Dmab should not be stopped at a lower BMD, in order to avoid more years of Dmab treatment and the subsequent risk of FXs upon discontinuation. However, our data suggest the need of further studies evaluating how to personalize the Dmab therapy duration on the basis of the individual FX risk. Indeed, the present study suggests that the same result in terms of net BMD gain at follow-up after Dmab discontinuation could be achieved more quickly and more safely with a brief course of Dmab. This is in line with a previous retrospective study by Everts-Graber and coauthors who found that a single Dmab injection followed by ZOL administration led to a greater BMD gain as compared with 2.5 years of Dmab therapy followed by ZOL administration (7, 30).

Finally, we reported that FX (patients 3, 4, and 5; Table 2) occurred in patients who received 2 ZOL infusions given 6 months apart, which is quite alarming. In the ProOff study, Everts-Graber and coauthors reported 9 patients, with Dmab therapy lasting more than 5 years, with incident VFX (of which 4 were multiple), but they did not specify the timing or if they had been treated with single or repeated ZOL (9). In our cohort, the only case of multiple VFX occurred in a woman before the first ZOL infusion was given, about 15 days later than the supposed date of Dmab administration. As these FX occurred very early after Dmab discontinuation, and we know that some patients may fracture even during Dmab or in case of delayed administration, we cannot be sure that such VFX was due to rebound rather than suboptimal response to Dmab or that a more precocious ZOL administration would have prevented that VFX (31). In the ZOLARMAB study, 2 patients had incident fragility VFX, both occurring in the first 3 months of Dmab washout, before ZOL was given. In spite of a not-demonstrated superiority in preserving BMD, this fact strengthens the ECTS suggestion to give ZOL 6 months after the last Dmab injection and not later (12).

Our study has some limitations. First, the retrospective design could have introduced some unknown bias. Second, the lack of a "control" group treated with a single infusion of ZOL prevented us from a direct comparison of the possible advantage of a repeated administration schedule addressed by CTX levels. Furthermore, we tried to administer the first ZOL 6 months after the last Dmab injection, but it was not

always possible due to the real-life organization; we did our best not to delay the infusion beyond 1 month. Lastly, we did not monitor CTX levels 3 months after the first ZOL infusion as recommended by the ECTS panel, and this approach could have delayed the second ZOL administration in some patients, preventing those subjects from the possible benefit of an early repeated treatment. Despite its limitations, our study also has strengths in that we report for the first time the outcome of the systematic application of the cut-off suggested by the ECTS for ZOL retreatment in patients discontinuing Dmab. In particular, our study highlights that up to 75% of patients discontinuing Dmab require repeated ZOL administration, and, still, it may not be sufficient in fully prevent rebound FX, which occurred in 9.6% of our patients, and BMD loss, which (on average) was significant in the group with not-suppressed CTX. Indeed, according to our data, the safety CTX levels to aim for might be lower than the cut-off indicated in the ECTS recommendations.

Besides the importance of maintaining bone turnover control, it seems crucial to guarantee BMD stability after Dmab withdrawal. However, the best strategy to achieve this result is still unknown, although it is clear that the use of BPs at standard doses for the treatment of osteoporosis is not enough in the majority of cases, and, as our study shows, even the administration of a second ZOL 6 months apart cannot guarantee the BMD maintenance in patients with persistently high CTX levels. It seems reasonable, therefore, to precisely administer the first ZOL 6 months after the last Dmab injection and to intensively start monitoring CTX levels immediately after in order to consider further ZOL administrations even earlier than 6 months.

Disclosures

The authors have nothing to disclose.

Data Availability

Some or 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, Wagman RB, Brandi ML, *et al.* 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. *Lancet Diabetes Endocrinol*. 2017;5(7):513-523.
2. Lacey DL, Boyle WJ, Simonet WS, *et al.* Bench to bedside: elucidation of the OPG–RANK–RANKL pathway and the development of denosumab. *Nat Rev Drug Discov*. 2012;11(5):401-419.
3. 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.
4. 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.
5. Zanchetta MB, Boailchuk J, Massari F, Silveira F, Bogado C, Zanchetta JR. Significant bone loss after stopping long-term denosumab treatment: a post FREEDOM study. *Osteoporos Int*. 2018;29(1):41-47.
6. Cummings SR, Ferrari S, Eastell R, *et al.* Vertebral fractures after discontinuation of denosumab: a post hoc analysis of the randomized placebo-controlled FREEDOM trial and its extension. *J Bone Miner Res*. 2018;33(2):190-198.
7. Cosman F, Huang S, McDermott M, Cummings SR. Multiple vertebral fractures after denosumab discontinuation: FREEDOM and FREEDOM extension trials additional post hoc analyses. *J Bone Miner Res*. 2022;37(11):2112-2120.
8. Ferrari S, Langdahl B. Mechanisms underlying the long-term and withdrawal effects of denosumab therapy on bone. *Nat Rev Rheumatol*. 2023;19(5):307-317.
9. Everts-Graber J, Reichenbach S, Gahl B, *et al.* Effects of zoledronate on bone mineral density and bone turnover after long-term denosumab therapy: observations in a real-world setting. *Bone*. 2022;163:116498.
10. Grassi G, Chiodini I, Palmieri S, Cairoli E, Arosio M, Eller-Vainicher C. Bisphosphonates after denosumab withdrawal reduce the vertebral fractures incidence. *Eur J Endocrinol*. 2021;185(3):387-396.
11. 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.
12. 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*. 2021;106(1):264-281.
13. 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.
14. 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.
15. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res*. 1993;8(9):1137-1148.
16. Kerkeni S, Kolta S, Fechtenbaum J, Roux C. Spinal deformity index (SDI) is a good predictor of incident vertebral fractures. *Osteoporos Int*. 2009;20(9):1547-1552.
17. Leder BZ, Tsai JN, Uihlein AV, *et al.* Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-switch study): extension of a randomised controlled trial. *Lancet*. 2015;386(9999):1147-1155.
18. McClung MR, Bolognese MA, Brown JP, *et al.* Skeletal responses to romosozumab after 12 months of denosumab. *JBMR Plus*. 2021;5(7):e10512.
19. Ramchand SK, David NL, Lee H, Eastell R, Tsai JN, Leder BZ. Efficacy of zoledronic acid in maintaining areal and volumetric bone density after combined denosumab and teriparatide administration: DATA-HD study extension. *J Bone Miner Res*. 2020;36(5):921-930.
20. 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.
21. Horne AM, Mihov B, Reid IR. Bone loss after romosozumab/denosumab: effects of bisphosphonates. *Calcif Tissue Int*. 2018;103(1):55-61.
22. Freemantle N, Satram-Hoang S, Tang ET, *et al.* 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.
23. Tutaworn T, Nieves JW, Wang Z, Levin JE, Yoo JE, Lane JM. Bone loss after denosumab discontinuation is prevented by alendronate and zoledronic acid but not risedronate: a retrospective study. *Osteoporos Int*. 2023;34(3):573-584.
24. Makras P, Appelman-Dijkstra NM, Papapoulos SE, *et al.* The duration of denosumab treatment and the efficacy of zoledronate to preserve bone mineral density after its discontinuation. *J Clin Endocrinol Metab*. 2021;106(10):e4155-e4162.

25. Liebich G, Lamy O, Aubry-Rozier B, Gonzalez-Rodriguez E. Maintenance of bone resorption markers in the low premenopausal range during the year following denosumab discontinuation is associated to bone density preservation. The ReoLaus study. *Bone*. 2023;172:116764.
26. Eastell R, Pigott T, Gossiel F, Naylor KE, Walsh JS, Peel NFA. DIAGNOSIS OF ENDOCRINE DISEASE: bone turnover markers: are they clinically useful? *Eur J Endocrinol*. 2018;178(1): R19-R31.
27. 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.
28. Sølling AS, Tsourdi E, Harsløf T, Langdahl BL. Denosumab discontinuation. *Curr Osteoporos Rep*. 2023;21(1):95-103.
29. McClung MR, Lippuner K, Brandi ML, *et al.* Effect of denosumab on trabecular bone score in postmenopausal women with osteoporosis. *Osteoporos Int*. 2017;28(10):2967-2973.
30. Everts-Graber J, Lehmann T. Bone mass gains after one denosumab injection followed by zoledronate. *J Clin Densitom*. 2022;25(3): 293-298.
31. Betella N, Biamonte E, Matarazzo C, *et al.* Suboptimal medication adherence may favor the progression of vertebral fractures in women with post-menopausal osteoporosis treated with denosumab. *Minerva Endocrinol*. 2020;45(3):165-171.