Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study

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ABSTRACT (250 words, maximum of 250 words)

Context: Discontinuation of denosumab leads to a rapid reversal of its therapeutic effect. However, there are no data regarding how unintended delays or missed injections of denosumab impact bone mineral density (BMD) response.

Objective: We examined the association of delays in injections of denosumab with BMD change.

Design: We used electronic medical records from two academic hospitals from 2010 to 2017.

Participants: Patients over 45 years of age and used at least two doses of 60mg denosumab. Denosumab adherence was evaluated by the medication coverage ratio (MCR). Good adherence corresponds to a dosing interval ≤7 months (defined by MCR ≥93%), moderate adherence corresponds to an interval of 7-10 months (MCR 75-93%), and poor adherence corresponds to an interval ≥10 months (MCR ≤74%).

Outcome Measures: Annualized percent BMD change from baseline at the lumbar spine, total hip, and femoral neck.

Results: We identified 938 denosumab injections among 151 patients; the mean (SD) age was 69 (10) years, and 95% were female. Patients with good adherence had an annualized BMD increase of 3.9% at the lumbar spine, compared with patients with moderate (3.0%) or poor adherence (1.4%, p for trend 0.002). Patients with good adherence had an annualized BMD increase of 2.1% at the total hip, compared with patients with moderate (1.3%) or poor adherence (0.6%, p for trend 0.002).

Conclusions: A longer interval between denosumab injections is associated with suboptimal BMD response at both spine and total hip. Strategies to improve the timely administration of denosumab in real-world settings are needed.

INTRODUCTION

Denosumab is an effective anti-resorptive drug commonly prescribed for the treatment of osteoporosis. It is a fully-humanized monoclonal antibody that binds to the receptor activator of the nuclear factor-κB ligand with high specificity and affinity, thereby impairing osteoclast function and inhibiting bone resorption(1). A large phase 3 randomized, placebo-controlled trial showed that denosumab 60 mg every six months significantly increased bone mineral density (BMD) over 24 months (2), and was associated with reduced vertebral, nonvertebral, and hip fractures at 36 months (3). A long-term extension trial showed that denosumab injections for up to 10 years was associated with low fracture incidence and sustained BMD increase without an obvious plateau(4).

Unlike bisphosphonates, discontinuation of denosumab leads to rapid reversal of its therapeutic effect(5); bone turnover rebounds above baseline levels three months after discontinuation(2), and BMD gained in the prior two years is reduced to baseline levels after one year without follow-on anti-osteoporosis treatment(2,6). Discontinuing denosumab also exposes patients to an increased risk of multiple vertebral fractures, particularly in patients with prior vertebral fractures (7–11). These fractures often occur within a very short off-treatment period (2 to 10 months after the denosumab therapeutic-effect has waned or 8-16 months from the last denosumab injection)(7), highlighting the importance of timely administration(7,12).

Although delaying or omitting denosumab doses is theoretically associated with unfavorable BMD response and increased fragility fracture risk, data from typical clinical practice are lacking. Previous studies mostly focused on the risk factors(13) and rate of denosumab discontinuation(13–17), which was 49% at 12 months and 64% at 24 months(14). In a European study, adherence (defined as < 7 months between two consecutive injections) was 83–89 % at 12 months and 63–70% at 24 months(13,15). While there is ample evidence that adherence to six-monthly dosing wanes, the impact of these delays on BMD is poorly defined (18).

A randomized controlled trial (RCT) is the gold standard for evaluating the efficacy of interventions. However, an RCT is not feasible in the case of denosumab dosing delay. An observational approach, which takes advantage of naturally occurring variations in the timing of denosumab administration, allows us to examine its impact on BMD response in routine clinical settings. In this study, we aim to examine the impact of denosumab delays on BMD.

METHODS

Data source

The Partners HealthCare electronic medical record (EMR) is used by several hospitals, including the Brigham and Women's Hospital and Massachusetts General Hospital. We used medical records of patients who took denosumab for the treatment of osteoporosis from October 2010 to December 2017. We first identified new users of denosumab who had been treated with this medication for at least 1 year (2 or more denosumab injections records). New users of denosumab and the date of injections were then verified by manual review of medical records.

Patients over 45 years of age and had at least two dual-energy X-ray absorptiometry (DXA) scans in the EMR system were included. The following exclusion criteria were then applied: a history of Paget's disease; simultaneous use of teriparatide, oral or intravenous bisphosphonates; and high-dosage denosumab (120 mg/ month) prescribed for cancer patients. The Partners HealthCare Institutional Review Board approved all aspects of this study (2017P001614).

Outcomes and study design

We adopted a repeated measures design to examine the impact of denosumab delays on BMD. Each subject may contribute to the analysis multiple times, depending on how many follow-up DXA tests were performed. The date of the first denosumab dose was defined as the index date. Follow-up

began after the index date and ended at any of the following events: switch to another anti-osteoporosis drug (bisphosphonates, teriparatide, or raloxifene), 9 months after the last dose, or the end of this study (December 31, 2017). Follow-up time for each patient was divided into a series of periods between each repeated DXA scan. We first defined a baseline DXA test window (2 years before and 3 months after the index date) and a follow-up DXA test window (6 months after index date to the last injection date plus 9 months) to identify baseline DXA and follow-up DXA. For patients with multiple DXAs within the baseline window, the scan closest to the index date was chosen as baseline DXA. Similarly, for patients with multiple DXAs within the follow-up window, the one closest to the end of the last dose's therapeutic effect (last dose plus 6 months) was chosen as the final DXA. Follow-up time was divided into a series of periods divided by two sequential DXA examinations (Supplement 1)(19); exposure (medication coverage ratio, MCR) and outcome (BMD change) were calculated for each period. The outcome of interest was annualized percent BMD change from baseline at the lumbar spine, total hip, and femoral neck (Supplement 1)(19). We used BMD (g/cm^2) from routine DXA scans (QDR 4500/4500A; Hologic, Bedford, MA) of the posteroanterior lumbar spine, total hip, and femoral neck.

Evaluation of denosumab adherence

We defined appropriate adherence as less than 7 months between two consecutive denosumab injections (16,17), which corresponds to a delay of <1 month for the subsequent dose. This definition is based on the known rapid reversibility of the suppression of bone resorption when denosumab is discontinued (15). Medication coverage ratio (MCR) was defined to quantitatively examine the association between the denosumab dosing interval and outcomes(13,15). The MCR measures the percentage of days that a patient was covered over a given time interval after receiving denosumab(20). We used the MCR as the parameter to define the study groups. Good MCR (≥93%) corresponds to a dosing interval ≤7 months, moderate MCR (75-93%) corresponds to 7-10 months, and poor MCR (≤74%) to ≥10 months. The MCR was calculated based on the assumption that each

injection of denosumab provides 180 days of therapeutic coverage and the effect of denosumab wanes after this period. We calculated the MCR in each interval between two DXA examinations. This approach can reflect the clinical situation that adherence may change over time. Sensitivity analysis was conducted using an alternative measure of denosumab delay, medication possession ratio (MPR), which additionally accounts for dosing before 6 months has elapsed (See supplement 2 for the illustration of the difference between MCR and MPR)(21). The dosage and date of each denosumab injection were verified by review of the medical record by one author (HL).

Covariate assessment

Patient characteristics were collected from the EMR. Variables of interest included age (at the index date), gender, race, body mass index (BMI), other medications of interest (hormone replacement therapy, raloxifene, glucocorticoids), and comorbidities (22). BMI was assessed based on the most recent value within a year prior to the index date. Comorbidities were defined using corresponding International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), or ICD-10-CM codes. We also collected information on bisphosphonate treatment duration and fragility fractures(23) occurring in the year before the index date.

Prior use of other anti-osteoporosis medications (alendronate, ibandronate, risedronate, zoledronic acid, and teriparatide) was verified by chart review and recorded. We classified drug brand or generic names into four categories, oral bisphosphonates (alendronate 10 mg/ day, or 70 mg/ week, ibandronate 150 mg/ month, risedronate 35 mg/ week), intravenous bisphosphonate (zoledronic acid 5 mg/ year or 2.5 mg/ 6 months, ibandronate 3 mg every 3 months, pamidronate 60 mg every 6 months or 30 mg every 3 months) and teriparatide (20 μg/ day percutaneous) for each patient. The duration of prior bisphosphonate-use was defined as the combined length of any oral or intravenous bisphosphonates.

Statistical analyses

Continuous variables were expressed as mean ± SD or median (interquartile range, IQR) when appropriate. Categorical variables were expressed as frequency and percentage. We described chronological adherence up to the 10th denosumab injection; that is, the proportion of patients who received the 2nd injection without delay, the proportion who received all injections up to the 3rd without delay, up to the 4th, 5th, etc. We then examined the association between MCR and annualized BMD change at the lumbar spine, total hip, and femoral neck. Since we performed the study at the subject-period level and one subject may contribute response data multiple times, we used generalized estimating equations (GEE) to analyze this correlated data. The robust sandwich estimate of the standard error was reported (24). Each observation (at subject-period level) was categorized into one of the three groups: poor (MCR ≤74%; corresponding to an interval of ≥10 months between two doses), moderate (MCR 75-93%; 7-10 months between two doses) and good (MCR ≥93%; within ≤7 months between two doses) based on the MCR calculated in each period. The poor adherence category served as the reference group. The models were adjusted for age, gender, BMI, rheumatoid arthritis, prior fragility fractures, prior alendronate, prior ibandronate, prior risedronate, prior intravenous bisphosphonate, prior bisphosphonates treatment length (months), prior anabolic treatment, prior glucocorticoid, length of the follow-up period, and the number of denosumab injections previously received.

Additionally, we performed a post hoc stratified analysis to exam the effect of timing of the dose delays (first 2 years vs. after 2 years of the therapy). We further examined interactions between MCR and other selected covariates (e.g., baseline BMD and cumulative denosumab duration). To aid interpretability, we used percentage change in BMD from baseline over each follow-up intervals(3,25–28). Annualized BMD increase was used to account for the different interval lengths. Predicted annualized BMD increases (average marginal means) in each category from multivariable models were reported.

In addition, six sensitivity analyses were performed for the primary GEE analyses. First, to more accurately capture the baseline BMD, we repeated the analyses using a strict baseline DXA window (less than 1 year prior to the index date). Second, given the fact the prior anabolic treatment may potentially lead to different BMD response to follow-up anti-resorptive agents, we repeated the analysis by excluding patients who received teriparatide before denosumab. Third, we restricted the analysis to female patients. Fourth, we excluded patients who received denosumab for <24 months to evaluate the long-term association of MCR and BMD changes. Fifth, given the concern that the rebound effect may be different between early time points (i.e., 1-4 months) and late time points (i.e., 12-24 months), we excluded observations with an off-treatment period > 12 months. Sixth, we repeated the analyses using MPR as an alternative measure of denosumab delay. Data were analyzed in the statistical environment R-3.5.0 (https://cran.r-project.org).

RESULTS

Patient demographics and baseline characteristics

We identified 151 patients who received at least two doses of denosumab, amounting to a total of 938 denosumab injections, between October 1, 2010, and December 31, 2017 (Figure 1). The mean (SD) age at index date was 69 (10) years, with 31% of patients being <65 years of age, 42% between 65 and 75 years, and 26% >75 years. The majority of patients were female (95%). 35% had a history of fragility fracture, 87% had a history of bisphosphonate-use, the average prior bisphosphate duration was 73 months, and 19% completed 2 years of prior teriparatide treatment (Table 1).

Denosumab adherence

Among 151 patients, the overall median (IQR) follow-up was 37 (28, 55) months. These patients received an average of six doses of denosumab; 15% received 2 to 3 doses, 62% received 4 to 8 doses, and 23% received over 8 doses. Overall, 21% of all administered denosumab injections were delayed by over one month, 83% patients received injections within the preferred interval for the 2nd

injection and dropped to 13% after 8th injection and 8% after 10th injection. 97% of patients received the next injection within 10 months for the 2nd dose, but only 40% did so after 10th injection, suggesting 60% patients would have at least one injected delayed for over 4 months at year 5 (Figure 2).

Two hundred and thirty-three follow-up DXA tests were identified from 151 patients, resulting in 233 short follow-up periods. The average length of these short follow-up periods was 21 months. MCR was calculated during each period and then used as a parameter for to group observations into good, moderate, poor adherence groups. The overall median MCR (IQR) calculated in each follow-up interval was 89% (85%, 97%), with 64% (49%, 71%) for poor adherence, 86% (83%, 90%) for moderate adherence, and 97% (96%, 99%) for good adherence group. The distribution of MCR is shown in Supplement 3(29).

Association between MCR and BMD response

Among all study subjects, unadjusted annualized percentage change in BMD was 2.7% (95% CI, 2.3 to 3.1%) at the lumbar spine, 1.2% (95% CI, 1.0 to 1.5%) at the total hip, and 1.1% (95% CI, 0.8 to 1.3%) at the femoral neck. Within the groups (poor, moderate and good adherence), unadjusted annualized change in BMD was 1.4%, 3.0% and 3.9%, respectively at the lumbar spine (p for trend = 0.002), and 0.6%, 1.3% and 2.1% respectively at the total hip (p for trend = 0.001). Annualized BMD change was not significantly different at the femoral neck across three groups: 1.3% poor, 1.5% moderate, and 1.7% good (p for trend = 0.49) (Table 2). Pairwise comparison showed that annualized BMD changes in good and moderate adherence groups were greater than those in poor adherence groups at both lumbar spine and total hip, but no significant difference of BMD changes were found across groups at the femoral neck (Supplement 4)(30).

In the final model adjusting for age, gender, BMI, rheumatoid arthritis, fragility fracture history, bisphosphonates history and duration, anabolic treatment history, glucocorticoid history, follow-up length and the number of denosumab injections previously received, annualized change in lumbar spine BMD was higher among subjects with good adherence (3.9%) than moderate adherence (3.0%) or poor adherence (1.4%) (p for trend 0.002). A similar trend was observed for total hip BMD (2.1% vs. 1.3% vs. 0.6%, p for trend = 0.002, Table 2). Femoral neck BMD changes were not associated with adherence (p for trend = 0.487). Pairwise comparisons from the adjusted model were similar to those from unadjusted models, differences in the annualized BMD increase at the total hip between the moderate adherence group and the poor group did not reach statistical significance (0.8%, 95%CI, -0.1 to 1.7%) (Supplement 4)(30).

In the multivariable model with a continuous measure for MCR, MCR was statistically significantly associated with annualized BMD changes at both the lumbar spine and total hip areas, but not for BMD changes at the femoral neck. In a post hoc stratified analysis, we compared the effect of delays during the first 2 years (first 4 doses) vs. after 2 years (5th or subsequent doses) (Table 3). The annualized BMD increase in the poor adherence group was consistently less than the moderate and good adherence groups, but all BMD increases were less dramatic in later years.

In the poor adherence group, the annualized BMD increase was 2.9% at the lumbar spine and 1.0% at the total hip during the first 2 years' treatment, while beyond 2 years, the increases were quite small, with only 0.1% at the lumbar spine and 0.1% at the total hip. But our study was underpowered to detect this effect modification; interactions between MCR and therapy length were not statistically significant. Effect size estimates from sensitivity analyses were consistent with the primary analysis (Figure 3). Since the BMD percentage scale might be influenced by the baseline chosen, we also repeated the same analysis with absolute BMD (g/cm^2); results were similar between the percentage and absolute BMD scales (data not shown).

DISCUSSION

In this study, we showed that adherence with denosumab injections over 3 to 5 years was suboptimal, and almost half of the study population experienced at least one injection-delay of over four months through 4 years. Our study demonstrates that longer intervals between denosumab administrations are associated with suboptimal BMD response at the total hip and lumbar spine. These results highlight the importance of timely denosumab administration when using this drug for long-term osteoporosis management.

An important observation of the current study is that adherence beyond 24 months declined dramatically, with proportions of adherent patients as low as 28% at 36 months, 13% at 48 months, and 8% at 60 months. A more disturbing finding is that a large proportion of the study population experienced at least one delay of over four months: as high as 27% at 36 months, 44% at 48 months, and 63% at 60 months. Given the observed difference in BMD increase among patients who received denosumab on schedule and those with >7 months between doses, these patients are likely at higher risk for suboptimal BMD improvements, or even decreases, during the off-treatment period. Interventions aimed at improving long-term adherence to denosumab should be implemented to achieve better treatment outcomes.

The consequences of the delays mentioned above were not well examined in previous studies. A prior study showed that the BMD responses did not differ significantly at one year among patients who received subsequent injection less than 5 months, between 5 and 7 months, or greater than 7 months after initial injection (18). However, the results of the current study showed that adherence was consistently associated with less robust improvements in annualized BMD response at both the lumbar spine and total hip. The inconsistent result may be explained by differences in study design.

Our study had longer follow-up with median 3 years and up to 5 years, evaluated adherence using time-varying MCR, and compared the BMD changes between the good (corresponding subsequent injection between 5 and 7 months) and poor adherence (corresponding subsequent injection greater than 10 months). Together, these factors gave us higher statistical power to detect differences in BMD change between patients with good and poor adherence. In previous randomized controlled trials, lumbar spine BMD increased by 3.2 to 6.7% and total hip BMD by 1.9 to 3.6% at 12 months(31). Although the estimated annualized BMD increase cannot be directly compared with that from trials, the low BMD response from the poor adherence group in contract to good adherence group suggests the effect of denosumab in poorly adherent populations were not fully achieved. A recent study by Bouxsein et al showed that greater improvements in BMD were strongly associated with greater reductions in vertebral and hip fractures(32): for a 2% improvement in total hip BMD, we might expect a 28% reduction in vertebral and 16% reduction in hip fracture risk. In the current study, we found a difference of 2.5% annualized BMD increase at the lumbar spine and 1.5% at total hip between adherent patients and non-adherent patients. These effect sizes may translate into considerable differences in fracture risk, but limitations of surrogate outcomes are well known, and further studies using fracture outcomes are needed.

In a post hoc stratified analysis, we checked whether the effect of delays was different between the early and later years of denosumab therapy. The annualized BMD increase in the poor adherence group was consistently less than moderate and good adherence groups, but all BMD increases were less in later years. In the poor adherence group, the annualized BMD increase was 2.9% at the lumbar spine and 1.0% at the total hip during the first 2 years treatment; beyond 2 years, the increases were quite small, with only 0.1% at the lumbar spine and 0.1% at the total hip. Delay might have a large effect in the later years than the early years, that is, rebound effects may be more substantial in later years. However, we did not have sufficient statistical power to detect the

interaction between delay and treatment duration. Studies using bone turnover markers or histomorphometric measurements may shed further light on this phenomenon.

In our study, the BMD response at the femoral neck was not sensitive to lower adherence, which deserves further discussion. One possible explanation is that BMD measurement at the femoral neck has much greater variability than that at the lumbar spine or total hip relative to the magnitude of BMD gains at each site, and thus may require a larger sample size to achieve statistical difference across MCR groups. Another hypothesis is that trabecular bone may be more vulnerable to intermittent bone resorption than cortical bone. Vertebral bone is up to 80-90% trabecular(33,34), the hip is about 60% trabecular(34) and at the femoral neck cortical bone prevails with only 25% being trabecular(33). The BMD change of trabecular bone might be the main contributor of BMD change difference across different MCR groups. As the femoral neck has relatively fewer trabecular, a much large sample size is needed to detect the difference across MCR groups. Future studies are needed to confirm these hypotheses.

Our study has several strengths. First, the longitudinal data allow us to quantitatively examine the impact of interventions that are difficult or unethical to experimentally manipulate. Using longitudinal DXA tests, both dosing delay and BMD changes can be accurately evaluated. Second, as denosumab is a long-acting agent, the gaps between injections can be accurately defined by MCR. We adopted a repeated measures design and measured MCR for each patient in a series of intervals to increase statistical power. Finally, our follow-up period was much longer than previous studies; the majority of existing studies have a follow-up duration of 1 to 2 years. Long-term assessment of denosumab adherence and its association with treatment outcomes, such as BMD, is an essential piece of information that supports the need for adherence over long-term treatment.

There were also limitations. We defined the baseline DXA using a relatively wide window (2 years before and 3 months after the first denosumab injection), but sensitivity analysis using a narrower window (1 year before and 3 months after the first injection) showed similar results. We required patients to have both a baseline and at least one follow-up DXA; this results in selection bias. A comparison of baseline characteristics showed that the included patients were younger and less frequently had fragility fractures compared to the excluded patients (Supplement 5)(35). Thus, results from the current study might underestimate the association between dosing delay and BMD response. In this study, we pooled the longitudinal BMD results from the DXA reports and cannot guarantee that these DXA tests were performed under exactly the same settings, but it is not likely to significantly change measurements since clinicians used these longitudinal BMDs to guide clinical decision making. Both MCR and MPR provide are good methods to examine the injection delay, but it requires an additional assumption. We assume that the off-treatment effect observed in serial follow-up intervals is similar and additive when the duration of the off-treatment period increases. Based on the pharmacodynamic profile of denosumab that the therapeutic-effect wanes quickly when discontinued, this is a reasonable but nevertheless strong assumption. In this study, we used annualized BMD change, which can be a helpful outcome for comparing BMD response across study groups. Since BMD increase is not linear, the magnitude of annualized BMD changes should not be viewed the same as the yearly BMD changes from trials, thus limiting the extrapolation of these estimates. Finally, we did not evaluate the difference in fracture endpoints due to low fracture incidence in the study cohort. Future studies with fracture endpoints are needed to confirm our results.

Conclusion and clinical implications

In conclusion, long-term adherence to denosumab was suboptimal in this routine clinical population. Better adherence was associated with greater annualized BMD response at both the lumbar spine and the total hip. The originality of this study is its evaluation of the impact of denosumab dosing

delay. Since denosumab administration requires an appointment with the health care system, delays may be unavoidable in routine clinical practice. Currently, little evidence exists regarding how long a delay must be avoided. Our results provide evidence that a delay of over four months (i.e.,>10 months between doses) may be unacceptable, but future studies are needed to determine the exact threshold. Determining effective strategies to improve adherence with denosumab, and implementing those strategies, are crucial if we are to optimize the therapeutic benefits of this highly effective therapy while minimizing potential adverse effects.

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AUTHOR CONTRIBUTIONS:

The authors met all the following conditions: (1) HL, BZL, and DHS conceived of and designed the work. (2) HL and CX acquired and analyzed the data, (3) HL, BZL, and DHS drafting the work. All authors (HL, KY, SKT, SZ, SUN, BZL, and DHS) revised the work for substantial intellectual contents and gave final approval of the version to submit. DHS controlled the decision to publish. DHS attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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REFERENCE

1. **Lacey DL, Boyle WJ, Simonet WS, Kostenuik PJ, Dougall WC, Sullivan JK, Martin JS, Dansey R.** Bench to bedside: elucidation of the OPG–RANK–RANKL pathway and the development of denosumab. *Nat. Rev. Drug Discov.* 2012;11(5):nrd3705.

2. **Bone HG, Bolognese MA, Yuen CK, Kendler DL, Miller PD, Yang Y-C, Grazette L, San Martin J, Gallagher JC.** 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.

3. **Cummings SR, Martin JS, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C.** Denosumab for Prevention of Fractures in Postmenopausal Women with Osteoporosis. *N. Engl. J. Med.* 2009;361(8):756–765.

4. **Bone HG, Wagman RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, Czerwiński E, Fahrleitner-Pammer A, Kendler DL, Lippuner K, Reginster J-Y, Roux C, Malouf J, Bradley MN, Daizadeh NS, Wang A, Dakin P, Pannacciulli N, Dempster DW, Papapoulos S.** 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.

5. **Anastasilakis AD, Polyzos SA, Makras P.** Denosumab vs bisphosphonates for the treatment of postmenopausal osteoporosis. *Eur. J. Endocrinol.* 2018;179(1):R31–R45.

6. **Miller PD, Bolognese MA, Lewiecki EM, McClung MR, Ding B, Austin M, Liu Y, San Martin J.** Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: A randomized blinded phase 2 clinical trial1*Edited by: Stuart Ralston. *Bone* 2008;43(2):222–229.

7. **Anastasilakis AD, Polyzos SA, Makras P, Aubry-Rozier B, Kaouri S, Lamy O.** Clinical Features of 24 Patients With Rebound-Associated Vertebral Fractures After Denosumab Discontinuation: Systematic Review and Additional Cases. *J. Bone Miner. Res.* 2017;32(6):1291–1296.

8. **Aubry-Rozier B, Gonzalez-Rodriguez E, Stoll D, Lamy O.** Severe spontaneous vertebral fractures after denosumab discontinuation: three case reports. *Osteoporos. Int.* 2016;27(5):1923–1925.

9. **Tsourdi E, Langdahl B, Cohen-Solal M, Aubry-Rozier B, Eriksen EF, Guañabens N, Obermayer-Pietsch B, Ralston SH, Eastell R, Zillikens MC.** Discontinuation of Denosumab therapy for osteoporosis: A systematic review and position statement by ECTS. *Bone* 2017;105(Supplement C):11–17.

10. **Popp AW, Zysset PK, Lippuner K.** Rebound-associated vertebral fractures after discontinuation of denosumab—from clinic and biomechanics. *Osteoporos. Int.* 2016;27(5):1917–1921.

11. **Cummings SR, Ferrari S, Eastell R, Gilchrist N, Jensen J-EB, McClung M, Roux C, Törring O, Valter I, Wang AT, Brown JP.** Vertebral Fractures After Discontinuation of Denosumab: A Post Hoc Analysis of the Randomized Placebo-Controlled FREEDOM Trial and Its Extension: VERTEBRAL FRACTURES AFTER DENOSUMAB DISCONTINUATION. *J. Bone Miner. Res.* 2017. doi:10.1002/jbmr.3337.

12. **Leaney A, Sztal-Mazer S.** Rebound vertebral fracture in the dental chair during a tooth extraction whilst on a treatment holiday from denosumab to avoid ONJ! *Bone* 2018;108:43.

13. **Fahrleitner-Pammer A, Papaioannou N, Gielen E, Feudjo Tepie M, Toffis C, Frieling I, Geusens P, Makras P, Boschitsch E, Callens J, Anastasilakis AD, Niedhart C, Resch H, Kalouche-Khalil L, Hadji P.** Factors associated with high 24-month persistence with denosumab: results of a real-world, non-interventional study of women with postmenopausal osteoporosis in Germany, Austria, Greece, and Belgium. *Arch. Osteoporos.* 2017;12(1). doi:10.1007/s11657-017-0351-2.

14. **Modi A, Sajjan S, Insinga R, Weaver J, Lewiecki EM, Harris ST.** Frequency of discontinuation of injectable osteoporosis therapies in US patients over 2 years. *Osteoporos. Int.* 2017;28(4):1355–1363.

15. **Hadji P, Papaioannou N, Gielen E, Feudjo Tepie M, Zhang E, Frieling I, Geusens P, Makras P, Resch H, Möller G, Kalouche-Khalil L, Fahrleitner-Pammer A.** Persistence, adherence, and medication-taking behavior in women with postmenopausal osteoporosis receiving denosumab in routine practice in Germany, Austria, Greece, and Belgium: 12-month results from a European non-interventional study. *Osteoporos. Int.* 2015;26(10):2479–2489.

16. **on behalf of the DAPS Investigators, Kendler DL, McClung MR, Freemantle N, Lillestol M, Moffett AH, Borenstein J, Satram-Hoang S, Yang Y-C, Kaur P, Macarios D, Siddhanti S.** Adherence, preference, and satisfaction of postmenopausal women taking denosumab or alendronate. *Osteoporos. Int.* 2011;22(6):1725–1735.

17. **on behalf of the DAPS Investigators, Freemantle N, Satram-Hoang S, Tang E-T, Kaur P, Macarios D, Siddhanti S, Borenstein J, Kendler DL.** 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.

18. **Wong-Pack M, Kalani A, Hordyk J, Ioannidis G, Bensen R, Bensen WG, Papaioannou A, Adachi JD, Lau AN.** The Effects of Noncompliance to Prolia (Denosumab) on the Changes in Bone Mineral Density: A Retrospective Review. *J. Osteoporos.* 2016;2016. doi:10.1155/2016/7903128.

19. **Lyu H, Zhao S, Yoshida K, Tedeschi SK, Xu C, Nigwekar SU, Leder BZ, Solomon DH.** Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study, Supplement 1. figshare 2019. Deposited 08 December 2019. doi:10.6084/m9.figshare.11340836.v2.

20. **Cramer JA, Roy A, Burrell A, Fairchild CJ, Fuldeore MJ, Ollendorf DA, Wong PK.** Medication Compliance and Persistence: Terminology and Definitions. *Value Health* 2008;11(1):44–47.

21. **Lyu H, Zhao SS, Yoshida K, Tedeschi SK, Xu C, Nigwekar SU, Leder BZ, Solomon DH.** Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study, Supplement 2. figshare 2019. Deposited 08 December 2019. doi:10.6084/m9.figshare.11340839.v1.

22. **Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi J-C, Saunders LD, Beck CA, Feasby TE, Ghali WA.** Coding Algorithms for Defining Comorbidities in ICD-9-CM and ICD-10 Administrative Data: *Med. Care* 2005;43(11):1130–1139.

23. **Solomon DH, Johnston SS, Boytsov NN, McMorrow D, Lane JM, Krohn KD.** Osteoporosis Medication Use After Hip Fracture in U.S. Patients Between 2002 and 2011. *J. Bone Miner. Res.* 2014;29(9):1929–1937.

24. **Hanley JA.** Statistical Analysis of Correlated Data Using Generalized Estimating Equations: An Orientation. *Am. J. Epidemiol.* 2003;157(4):364–375.

25. **Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster J-Y, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mellström D, Oefjord ES, Marcinowska-Suchowierska E, Salmi J, Mulder H, Halse J, Sawicki AZ, Mitlak BH.** Effect of Parathyroid Hormone (1-34) on Fractures and Bone Mineral Density in Postmenopausal Women with Osteoporosis. *N. Engl. J. Med.* 2001;344(19):1434–1441.

26. **Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC, Man Z, Mautalen C, Mesenbrink P, Hu H, Caminis J, Tong K, Rosario-Jansen T, Krasnow J, Hue TF, Sellmeyer D, Eriksen EF, Cummings SR.** Once-Yearly Zoledronic Acid for Treatment of Postmenopausal Osteoporosis. *N. Engl. J. Med.* 2007;356(18):1809–1822.

27. **Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM.** The Effects of Parathyroid Hormone, Alendronate, or Both in Men with Osteoporosis. *N. Engl. J. Med.* 2003;349(13):1216–1226.

28. **McClung MR, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH, Peacock M, Miller PD, Lederman SN, Chesnut CH, Lain D, Kivitz AJ, Holloway DL, Zhang C, Peterson MC, Bekker PJ.** Denosumab in Postmenopausal Women with Low Bone Mineral Density. *N. Engl. J. Med.* 2006;354(8):821–831.

29. **Lyu H, Zhao S, Yoshida K, Tedeschi SK, Xu C, Nigwekar SU, Leder BZ, Solomon DH.** Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study, Supplement 3. figshare 2019. Deposited 08 December 2019. doi:10.6084/m9.figshare.11340842.v1.

30. **Lyu H, Zhao S, Yoshida K, Tedeschi SK, Xu C, Nigwekar SU, Leder BZ, Solomon DH.** Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study, Supplement 4. figshare 2019. Deposited 08 December 2019. doi:10.6084/m9.figshare.11340848.v1.

31. **Lyu H, Jundi B, Xu C, Tedeschi SK, Yoshida K, Zhao S, Nigwekar SU, Leder BZ, Solomon DH.** Comparison of denosumab vs. bisphosphonates in osteoporosis patients: A meta-analysis of randomized controlled trials. *J. Clin. Endocrinol. Metab.* 2018:21.

32. **Bouxsein ML, Eastell R, Lui L-Y, Wu LA, de Papp AE, Grauer A, Marin F, Cauley JA, Bauer DC, Black DM, for the FNIH Bone Quality Project.** Change in Bone Density and Reduction in Fracture Risk: A Meta-Regression of Published Trials: CHANGE IN BMD AND FRACTURE RISK REDUCTION. *J. Bone Miner. Res.* 2019;34(4):632–642.

33. **Felix W. W.** Role of Cortical and Trabecular Bone Architecture in Osteoporosis (PDF).

34. **Eastell R, O'Neill TW, Hofbauer LC, Langdahl B, Reid IR, Gold DT, Cummings SR.** Postmenopausal osteoporosis. *Nat. Rev. Dis. Primer* 2016;2:16069.

35. **Lyu H, Zhao S, Yoshida K, Tedeschi SK, Xu C, Nigwekar SU, Leder BZ, Solomon DH.** Delayed Denosumab Injections and Bone Mineral Density Response: An Electronic Health Record-based Study, Supplement 5. figshare 2019. Deposited 08 December 2019. doi:10.6084/m9.figshare.11340851.v1.

Figure legends

Figure 1 Study population

Figure 2 Adherence of denosumab from 2nd to 10th injections Adherence was examined using different adherence window (30 days and 120 days), chronological adherence was high at 2nd injection, but dropped dramatically.

Figure 3 Results of sensitivity analyses.

Comparisons between primary analysis and six sensitivity analyses for BMD increase at the lumbar spine, total hip, and femoral neck. Primary result: Annualized BMD changes of the three study groups from the primary analyses; Use 1-year baseline window: repeat the analyses using a strict baseline DXA window (less than 1 year prior to the index date); No prior teriparatide: repeat the analysis by excluding patients who received teriparatide before denosumab; Females only: restrict the analyses to female patients; DMAb over 24 months: excluded patients who received denosumab for <24 months; Alternative measurement MPR: repeat the analyses using MPR; and Off-treatment period within 12 months: exclude observations with an off-treatment period > 12 months.

The sensitivity analyses were adjusted for age, gender, BMI, rheumatoid arthritis (Yes/No), prior fragility fractures (Yes/No), prior alendronate (Yes/No), prior ibandronate (Yes/No), prior risedronate (Yes/No), intravenous bisphosphonate (Yes/No), prior bisphosphonates treatment length (months), prior anabolic treatment (Yes/No), prior glucocorticoid (Yes/No), length of follow-up period, and the number of denosumab injections previously received;

p value is from a trend test of an ordered relationship across the three groups (poor, moderate and good); DMAb, denosumab.

Table 1 Baseline characteristics of the study subjects

	Poor adherence MCR (<74%)	Moderate adherence MCR (75-93%)	Good adherence MCR (>93%)	p
N [a]	31	67	135	
Demographics				
Age, mean (SD), years	67 (11)	69 (11)	69 (10)	0.520
Female, %	31 (100.0)	62 (92.5)	129 (95.6)	0.262
BMI, mean (SD), kg/m2	23.99 (4.03)	24.34 (5.03)	23.58 (3.84)	0.478
Comorbidities, %				
Hyperthyroidism	1 (3.2)	8 (11.9)	11 (8.1)	0.345
Esophagus disease	17 (54.8)	36 (53.7)	64 (47.4)	0.600
Hypertension	15 (48.4)	42 (62.7)	75 (55.6)	0.383
Myocardial infarction	1 (3.2)	1 (1.5)	7 (5.2)	0.431
Peripheral vascular disease	0 (0.0)	4 (6.0)	13 (9.6)	0.157
Chronic pulmonary disease	15 (48.4)	22 (32.8)	53 (39.3)	0.330
Diabetes	6 (19.4)	13 (19.4)	27 (20.0)	0.993
Peptic ulcer disease	3 (9.7)	4 (6.0)	3 (2.2)	0.132
Chronic kidney diseases	7 (22.6)	13 (19.4)	29 (21.5)	0.919
Rheumatoid arthritis	1 (3.2)	6 (9.0)	9 (6.7)	0.575
Osteoarthritis	21 (67.7)	39 (58.2)	79 (58.5)	0.615
Any cancer	17 (54.8)	25 (37.3)	66 (48.9)	0.178
CCI Index (median [IQR])	3 [2, 6]	2 [0, 5]	2 [0, 5]	0.137
Baseline BMD, mean (SD), g/cm²				
Lumbar spine	0.81 (0.11)	0.78 (0.11)	0.78 (0.10)	0.403
Total hip	0.74 (0.07)	0.70 (0.11)	0.72 (0.08)	0.109
Femoral neck	0.60 (0.07)	0.58 (0.09)	0.59 (0.08)	0.551
Prior fracture, %				
Fragility fracture	12 (38.7)	22 (32.8)	46 (34.1)	0.846
Hip fracture	1 (3.2)	7 (10.4)	6 (4.4)	0.188

Spine fracture	4 (12.9)	11 (16.4)	25 (18.5)	0.742
Medications, ever use, %				
Alendronate	20 (64.5)	47 (70.1)	88 (65.2)	0.756
Ibandronate	1 (3.2)	4 (6.0)	12 (8.9)	0.487
Risedronate	5 (16.1)	7 (10.4)	18 (13.3)	0.715
IV bisphosphonate	9 (29.0)	22 (32.8)	43 (31.9)	0.931
BP length, mean (SD), months	66 (48)	71(50)	74 (51)	0.725
Teriparatide	6 (19.4)	13 (19.4)	32 (23.7)	0.734
Systemic corticosteroids	22 (71.0)	41 (61.2)	78 (57.8)	0.396
Hormone replacement therapy	20 (64.5)	26 (38.8)	52 (38.5)	0.025
Raloxifene	6 (19.4)	3 (4.5)	24 (17.8)	0.026
Labs				
Serum Vit D, mean (SD), ng/mL	42.97 (15.88)	40.33 (12.01)	45.11 (14.60)	0.078
Serum Ca^{2+}, mean (SD), mg/dL	9.52 (0.36)	9.63 (0.42)	9.68 (0.50)	0.226
Serum creatinine, mean (SD), mg/dL	0.92 (0.39)	0.99 (0.47)	0.98 (0.40)	0.790
eGFR, mean (SD), mL/min/1.73 m^2	54.48 (11.25)	52.79 (11.95)	53.50 (11.73)	0.797

[a] N was calculated at an observational level; each individual may contribute to different adherence groups more than one time; BMI, body mass index; SD, standard deviation; CCI, Charlson Comorbidity Index; IV, intravenous.

Table 2. Annualized percentage change in BMD across three groups

Models	Annualized BMD increase from baseline % [a] (95%CI)			p for trend
	Poor adherence MCR (<74%)	Moderate adherence MCR (75-93%)	Good adherence MCR (>93%)	
Unadjusted				
Lumbar spine	1.4 (0.3, 2.5)	3.0 (2.3, 3.7)	3.9 (3.0, 4.8)	0.002
Total hip	0.6 (-0.2, 1.3)	1.3 (1.0, 1.7)	2.1 (1.6, 2.6)	0.001
Femoral Neck	1.3 (0.3, 2.3)	1.5 (0.9, 2.0)	1.7 (0.9, 2.6)	0.491
Adjusted [b]				
Lumbar spine	1.4 (0.5, 2.3)	3.0 (2.3, 3.6)	3.9 (3.1, 4.7)	0.002
Total hip	0.6 (-0.2, 1.3)	1.3 (1.0, 1.6)	2.1 (1.6, 2.6)	0.002
Femoral Neck	1.3 (0.2, 2.3)	1.5 (0.9, 2.0)	1.7 (0.9, 2.6)	0.487

[a] We performed the study at the subject-period level, and one subject may contribute response data multiple times. The average marginal means were used. The average marginal effect means are the average fitted values of the annualized percentage change in BMD for each of the three categories from the regression model. MCR, medicine coverage ratio.

[b] Adjusted for age, gender, BMI, rheumatoid arthritis (Yes/No), prior fragility fractures (Yes/No), prior alendronate (Yes/No), prior ibandronate (Yes/No), prior risedronate (Yes/No), intravenous bisphosphonate (Yes/No), prior bisphosphonates treatment length (months), prior anabolic treatment (Yes/No), prior glucocorticoid (Yes/No), length of follow-up period, and the number of denosumab injections previously received.

Table 3 Delayed effect on annualized BMD increase in the early years versus later years.

Adjusted models [b]	Annualized BMD increase from baseline % [a] (95%CI)			p for trend
	Poor adherence MCR (<74%)	Moderate adherence MCR (75-93%)	Good adherence MCR (>93%)	
In the early years (cumulative DMAb ≤ 4 doses)				
Lumbar spine	2.9 (1.6, 4.2)	3.2 (2.3, 4.2)	4.4 (3.3, 5.4)	0.100
Total hip	1.0 (-0.1, 2.1)	1.7 (1.3, 2.2)	2.6 (1.9, 3.2)	0.003
Femoral Neck	2.5 (1.0, 4.0)	1.9 (1.2, 2.6)	2.2 (1.1, 3.3)	0.716
In later years (cumulative DMAb ≥ 5 doses)				
Lumbar spine	0.1 (-0.8, 1.0)	2.6 (1.8, 3.3)	2.4 (1.7, 3.1)	0.004
Total hip	0.1 (-0.7, 1.0)	0.7 (0.3, 1.2)	0.8 (0.5, 1.2)	0.157
Femoral Neck	0.05 (-0.8, 0.9)	0.7 (-0.2, 1.6)	0.6 (-0.04, 1.2)	0.558

[a] The average marginal means were used.

[b] Adjusted by age, gender, BMI, rheumatoid arthritis (Yes/No), prior fragility fractures (Yes/No), prior alendronate (Yes/No), prior ibandronate (Yes/No), prior risedronate (Yes/No), intravenous bisphosphonate (Yes/No), prior bisphosphonates treatment length (months), prior anabolic treatment (Yes/No), prior glucocorticoid (Yes/No), length of follow-up period, and the number of denosumab injections previously received.

Figure 1

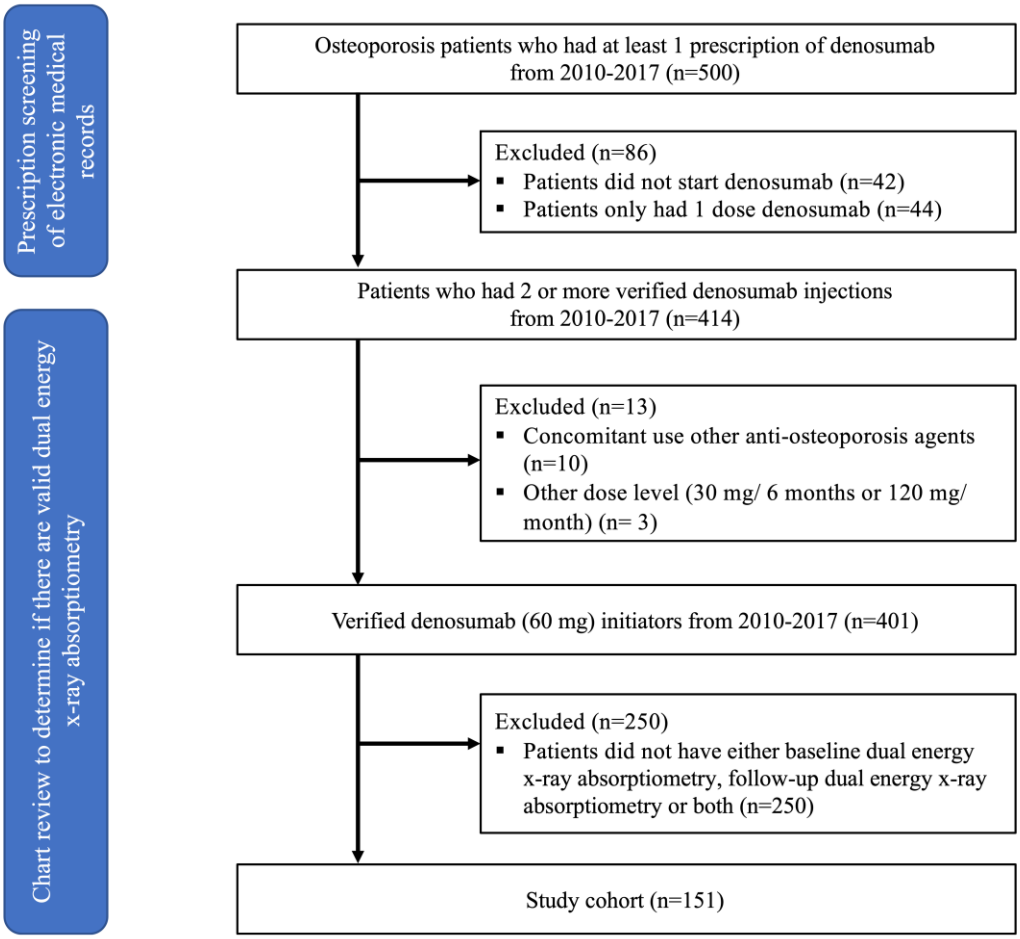

Figure 2

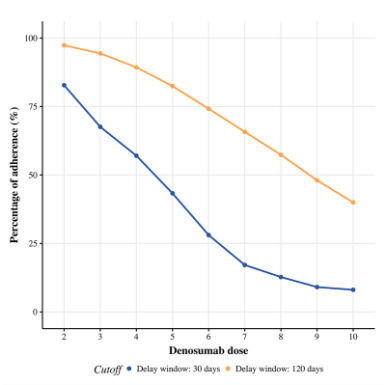

Figure 3