Delayed Denosumab Injections and Fracture Risk Among Patients With Osteoporosis

A Population-Based Cohort Study

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Background: Denosumab is effective for osteoporosis, but discontinuation leads to rapid reversal of its therapeutic effect.

Objective: To estimate the risk for fracture among users of denosumab who delayed subsequent doses compared with users who received doses on time.

Design: Population-based cohort study.

Setting: The Health Improvement Network U.K. primary care database, 2010 to 2019.

Patients: Persons aged 45 years or older who initiated denosumab therapy for osteoporosis.

Measurements: Observational data were used to emulate an analysis of a hypothetical trial with 3 dosing intervals: subsequent denosumab injection given within 4 weeks after the recommended date ("on time"), delay by 4 to 16 weeks ("short delay"), and delay by more than 16 weeks ("long delay"). The primary outcome was a composite of all fracture types at 6 months after the recommended date. Secondary outcomes were major osteoporotic fracture, vertebral fracture, hip fracture, and nonvertebral fracture.

Results: Investigators identified 2594 patients initiating denosumab therapy. The risk for composite fracture over 6 months was 27.3 in 1000 for on-time dosing, 32.2 in 1000 for short delay, and 42.4 in 1000 for long delay. Compared with on-time injections, short delay had a hazard ratio (HR) for composite fracture of 1.03 (95% CI, 0.63 to 1.69) and long delay an HR of 1.44 (CI, 0.96 to 2.17) (*P* for trend = 0.093). For vertebral fractures, short delay had an HR of 1.48

(CI, 0.58 to 3.79) and long delay an HR of 3.91 (CI, 1.62 to 9.45).

Limitation: Dosing schedules were not randomly assigned.

Conclusion: Although delayed administration of subsequent denosumab doses by more than 16 weeks is associated with increased risk for vertebral fracture compared with on-time dosing, evidence is insufficient to conclude that fracture risk is increased at other anatomical sites with long delay.

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Denosumab is an effective antiresorptive drug prescribed for the treatment of osteoporosis (1). Discontinuation of denosumab therapy, however, results in accelerated bone turnover, rapid loss of bone mineral density (BMD), and an increased rate of multiple vertebral fractures, often within a short off-treatment period of 2 to 10 months (that is, 8 months after the prior denosumab injection) (2–9). A post hoc analysis of a randomized placebo-controlled trial and its extension reported that withdrawing denosumab without switching to another antiresorptive agent was associated with higher risk for multiple vertebral fractures in the subsequent year (8). Another observational study showed an even more alarming reversal of benefit after denosumab therapy was stopped (10), which was associated with higher risk for vertebral fracture (incidence rate ratio [RR], 4.7 [95% CI, 2.3 to 9.6]), major osteoporotic fracture (RR, 3.2 [CI, 2.2 to 4.8]), and hip fracture (RR, 5.3 [CI, 2.0 to 13.9]) in the year after discontinuation. However, whether delaying subsequent injections beyond the recommended 6-month interval is associated with fractures is unknown.

Delayed dosing of denosumab is very common in routine clinical practice (11–13). Among patients who had been using denosumab for several years, almost 50% had at least 1 injection delay of more than 4 months. Delaying the subsequent dose by several months is associated with reduced improvement in BMD (11). However, to our knowledge, no study has yet examined

the effect of denosumab delay on fracture risk.

Because it may not be ethical to do a randomized controlled trial to address this question, we emulated an analysis of a hypothetical trial using observational data to examine the causal effect of delayed denosumab injection on fracture risk (14, 15).

Methods

Study Population and Design

We used The Health Improvement Network (THIN), an electronic database of medical records from primary care practitioners in the United Kingdom, as the data source. This database contains health information on approximately 17 million patients from 790 general practices (16). For this study, we extracted data on demographic characteristics, diagnosis, and medications from 2010 to 2019.

Through this period, primary care data were coded using the Read classification system, which we used to identify relevant diagnoses. The Health Improvement Network uses a drug dictionary based on data from the Multilex classification system (17). The scientific review committee of THIN approved this study (19THIN079), and informed consent was waived.

This retrospective cohort study takes advantage of naturally occurring variation in the timing of denosumab administration, allowing us to examine the effect of this variation on fracture risk in routine clinical settings. We emulate a hypothetical randomized controlled trial (the "target trial") comparing 3 dosing intervals for subsequent denosumab injections (on time, short delay, and long delay) using observational data (Appendix Table 1, available at Annals.org) (18). Target trial emulation is a design framework for observational research that reduces bias and better aligns results with those of actual randomized controlled trials (14). Appendix Table 1 provides details on the specification of the target trial and its emulation using observational data.

Study Cohort

This study included injection data from persons aged 45 years or older who initiated denosumab therapy for the management of osteoporosis between 2010 and 2019. We identified denosumab dosages of 60 mg subcutaneously every 6 months. We excluded persons who had only 1 denosumab prescription record, received the subsequent injection within 150 days after the prior dose, or also used any other antiosteoporosis drugs (estrogen, selective estrogen receptor modulators, alendronate, risedronate, ibandronate, zoledronic acid, other bisphosphonates, or teriparatide). Patients were censored when they switched to another potent antiosteoporosis medication or discontinued denosumab therapy (6 months after the last dose).

To improve statistical efficiency, we did the study at the injection level; that is, each individual could contribute multiple denosumab doses. We used a 6-month "run-in" period (a concept borrowed from randomized controlled trials) to assess the eligible injections, which should follow a prior dose. Eligible patients should not switch to other antiosteoporosis medications and should be alive at the time of follow-up. At the end of the run-in period, a subsequent dose should be given and may raise concerns if delayed. We examined the effect of delaying the subsequent dose on fracture risk in the 6 months after the run-in period (Appendix Figure 1, available at Annals.org). Under the assumption that the effect of delay was similar among the second, third, and fourth injections, we pooled their effect estimates. In accordance with the clinical practice that patients who have fractures while receiving denosumab are still eligible for the treatment, we did not exclude patients who had fractures in the run-in period.

We set the recommended date of a subsequent dose of denosumab as 6 months after a prior dose; thus, it would be given at the end of the run-in period. We compared the effect of various delays of the subsequent dose (Appendix Figure 1): "on time," or within 4 weeks after the recommended date; "short delay," or between 4 and 16 weeks after the recommended date; and "long delay," or more than 16 weeks after the recommended date but not beyond 6

months (those who had not received a subsequent dose by the end of follow-up were also classified as having a long delay). In clinical practice, patients who receive a subsequent dose "early" (that is, 5 to 6 months after the prior dose) are also viewed as having it on time; thus, we classified these injections as "on time." A sensitivity analysis excluding these injections did not substantially change the estimates.

Fracture Outcomes

We compared fracture risk among the 3 groups during the 6-month follow-up, with a specific interest in the fracture trend difference. The primary outcome of interest was composite fracture, which included all types of fracture. Secondary outcomes were major osteoporotic fracture (hip, vertebral, wrist, humerus, pelvis, and rib), vertebral fracture, hip fracture, and nonvertebral fracture (19, 20). We defined fracture outcomes using validated Read codes (19, 21, 22); positive predictive value was 88.1% for vertebral fracture and 91.0% for hip fracture (22–24). Follow-up started the day after the end of the run-in period (Appendix Figure 1) and continued until the first of any of the following: a first fracture event, death, a switch to any other antiosteoporosis drug, 6 months, or the end of the study (30 April 2019).

Covariates

We set the recommended date of a subsequent dose (6 months after a prior dose) as the index date and used a 2-year baseline window to pool data for the following variables (Appendix Figure 1): sociodemographic factors (for example, age and sex), lifestyle factors (for example, smoking and alcohol use), comorbid conditions that are potential risk factors for fracture, medications that affect bone metabolism, and antiosteoporosis therapy history (alendronate, ibandronate, risedronate, zoledronic acid, teriparatide, other bisphosphonates, and hormone replacement therapy). Charlson Comorbidity Index score was calculated with a Read coding algorithm (25). Ten-year risk for osteoporotic fracture was calculated using the QFracture 2012 algorithm (26). In addition, we pooled time-varying covariates measured after baseline at 1-week

intervals (Supplement, available at Annals.org).

Statistical Analysis

We explicitly emulated a randomized controlled trial comparing 3 groups with various delays of subsequent injection. Because the exposure of interest was "exposure duration" (on time vs. short delay vs. long delay), we adopted a "cloning, censoring, and weighting" approach to estimate the effect of delay duration on time to fracture using observational data (27). We created a data set with 3 copies of each eligible injection at baseline and assigned each of the replicates to 1 group at the start of follow-up (Appendix Figure 2, available at Annals.org). We censored replicates if and when they deviated from the assigned group, ensuring that replicates follow their assigned group. However, doing so has the potential to introduce selection bias, which we accounted for using time-varying inverse probability weights (27, 28). The denominator of the inverse probability weight was the probability that a replicate received his or her own observed treatment given his or her covariate history at and after baseline, treatment history, and not having a fracture before time *t*. In addition, we assigned a second time-varying inverse probability weight to address the imbalance in other reasons for loss to follow-up. These weights created 3 pseudopopulations in which treatment is independent of prognostic factor history (the Supplement provides technical details of this method). Differences in fracture events between the 3 pseudopopulations approximated the effect of delays of subsequent injections on fracture.

For the relative estimates, we fitted a pooled logistic regression model for each outcome, adjusting for baseline confounders in the weighted population (29). Because the outcome was rare, the odds ratio from this model approximated the hazard ratio (HR) (28, 30). The linear trend of group difference was tested with a continuous indicator of the delay group in the same model. We used a robust SE to compute conservative 95% CIs for all of the HR estimates. We also estimated absolute risks by fitting the pooled logistic models with product ("interaction")

terms between the delay indicator and the week of follow-up variables. The models' predicted values were then used to estimate the cumulative incidence of fractures from baseline. The cumulative incidence curves were standardized to the baseline variables (15). We used a nonparametric bootstrap with 500 samples to compute the 95% CIs for absolute estimates.

To test the robustness of the primary analysis, we did several sensitivity analyses (1). In the primary analysis, patients who had a fracture during the run-in period were eligible. However, patients with recent fractures have high risk for further fractures and could be more adherent to denosumab. The primary analysis could potentially underestimate the fracture risk difference between long delay and on time; therefore, we repeated the analysis, excluding patients who had fractures during the run-in period (2). Unmeasured confounding (for example, vitamin D or calcium, diet, and activity level) may bias the estimates from this observational study; we examined this bias using the E-value (31). We did predefined subgroup analyses stratified by age, prior duration of bisphosphonate use, and baseline fracture risk to examine fracture risk among study groups with different characteristics. In addition, to better evaluate the association between injection delay and fracture risk, we did an exploratory analysis in which we extended 3 groups to 23 groups, representing delay of a subsequent dose by 4 to 26 weeks at 1-week increments. Then, we estimated the delay effect by smoothing over the 23 groups using a flexible fourth-degree polynomial function (32–34). This analysis allowed us to assess the continuous relationship between denosumab injection delay and fracture risk.

All *P* values were 2-sided, and $P < 0.05$ was considered statistically significant for all tests. All analyses were done using R, version 3.5.2 (R Foundation), and SAS, version 9.4 (SAS Institute).

Role of the Funding Source

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conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. The authors had complete control over the design, conduct, and reporting of the study.

Results

Study Cohort

We identified 3964 persons who initiated denosumab treatment for osteoporosis between 1 June 2010 and 30 April 2019. After excluding 1370 of these 3964 initiators who did not complete the run-in period, we had a final cohort of 2594 patients. These patients contributed a total of 6144 qualified denosumab injections (Figure 1) that were used to emulate the target trial; 1856 of these injections were administered within 4 weeks before the index date (thus belonging only to the on-time group), leaving 4288 injections that could be delayed. The study population had a low burden of comorbid conditions, but participants were at very high risk for fracture, with a mean 10-year risk for major osteoporotic fracture of 22% (Table 1). The mean age at baseline was 76 years (SD, 10). Most patients were female (94%), 53% had a history of major osteoporotic fracture, 19% had a history of hip fracture, and 15% had had a vertebral fracture.

Comparative Fracture Risk

The cumulative risk for composite fracture overlapped for on-time dosing, short delay, and long delay (Figure 2); it was 27.3 in 1000 for on time, 32.2 in 1000 for short delay, and 42.4 in 1000 for long delay over 6 months. The corresponding risk difference between short delay and on-time dosing over 6 months was 4.8 (CI, −1.2 to 10.1) in 1000, and the HR was 1.03 (CI, 0.63 to 1.69). The corresponding risk difference between long delay and on-time dosing over 6 months was 15.0 (CI, 1.4 to 33.5) in 1000, and the HR was 1.44 (CI, 0.96 to 2.17). The *P* value for the composite fracture trend difference across the 3 groups was 0.093 (Table 2). The cumulative risk

for vertebral fracture over 6 months was 2.2 in 1000 for on-time dosing, 3.6 in 1000 for short delay, and 10.1 in 1000 for long delay. The corresponding risk difference between short delay and on-time dosing over 6 months was 1.4 (CI, −0.6 to 2.4) in 1000, and the HR was 1.48 (CI, 0.58 to 3.79). The corresponding risk difference between long delay and on-time dosing over 6 months was 7.9 (CI, 1.1 to 16.6) in 1000, and the HR was 3.91 (CI, 1.62 to 9.45). Results of the 4 secondary outcomes are shown in Table 2, Figure 2, and Appendix Figure 3 (available at Annals.org). A borderline increase in fracture risk with long delay was seen for major osteoporotic fracture, but not for hip or nonvertebral fractures (Table 2).

Results of sensitivity analyses were similar to those of the primary analysis. For vertebral fracture, short delay was associated with a similar fracture risk to on-time dosing; long delay had a higher risk than on-time dosing (Appendix Table 2, available at Annals.org). In an analysis restricted to patients who did not have fractures in the 6 months before the start of follow-up, long delay had a higher risk for fracture than on-time dosing for composite fracture (HR, 1.58 [CI, 1.04 to 2.41]), major osteoporotic fracture (HR, 2.00 [CI, 1.18 to 3.39]), and vertebral fracture (HR, 4.61 [CI, 1.78 to 11.9]) (Table 2). Although sensitivity analyses were somewhat underpowered for composite, hip, and nonvertebral fracture, that for vertebral fracture suggested that long delay had a higher risk for fracture than on-time dosing, with HRs ranging from 3.92 to 6.32 (Appendix Table 2). To examine the effect of unmeasured confounding, we found an E-value of 7.28 with a lower confidence limit of 2.62 for vertebral fracture, meaning that substantial unmeasured confounding would be needed to explain away the association between long delay and vertebral fracture risk to the null. The E-value for composite fracture was 2.23, with a lower confidence limit of 1.00 (Appendix Table 3, available at Annals.org). This suggests a more robust causal relationship between long delay and vertebral fracture than between long delay and composite fracture. We found no statistically significant effect modifier when we examined interactions with various patient characteristics (Table 3). In an exploratory

analysis examining the continuous dose–response relationship between delay and fracture risk (Appendix Figure 4, available at Annals.org), smoothed curves confirmed that risk for fracture increased with delay duration.

Discussion

Delaying subsequent denosumab doses by more than 4 months was associated with increased risk for vertebral fracture compared with on-time injection; however, evidence was insufficient to conclude that fracture risk was increased at other anatomical sites with long delay. This study suggests the importance of timely denosumab administration when used for long-term osteoporosis management. Currently, the Endocrine Society (35) and National Osteoporosis Foundation (36) recommend that “administration of denosumab should not be delayed or stopped without subsequent antiresorptive" (35). However, this recommendation is based on an ungraded good practice statement. Our study provides supportive evidence that poor adherence is associated with less benefit from denosumab.

In a previous study, we examined the effect of poor adherence to 6-monthly dosing with denosumab on BMD response (11). Patients with good adherence (equivalent to on time) had an average BMD increase of 3.9% at the lumbar spine, higher than patients with moderate adherence (equivalent to short delay; 2.8%) or poor adherence (equivalent to long delay; 1.6%) (*P* for trend = 0.009). The association was similar for total hip BMD. These results are compatible with the current study using fracture end points. Patients who delayed their subsequent dose of denosumab by more than 4 months had a 3.91-fold increased risk for vertebral fracture compared with those who received it on time.

Denosumab delay can be viewed as temporary discontinuation; thus, the effect of denosumab delay on fracture shares the same mechanism as discontinuation. A previous pharmacokinetic study showed that serum denosumab concentration reached its highest level

immediately after administration and gradually decreased to less than 100 ng/mL at 6 months (37). At the same time, bone resorption biomarker urinary *N*-telopeptides of type I collagen–creatinine ratio started to increase at 6 months and returned to the baseline level around 8 to 9 months in healthy participants (38). However, in the osteoporosis population, bone turnover markers increased above the baseline level after 3 months of discontinuation (2). In terms of fracture risk, Appendix Figure 4 shows that risk may increase around 10 to 12 weeks after the recommended date of the subsequent dose.

Fracture associated with denosumab withdrawal was first reported in 2016 (4–8), raising concerns about increased risk for multiple vertebral fractures after stopping denosumab therapy. In a clinical trial population who discontinued denosumab, the vertebral fracture rate increased about 5-fold, from 1.2 per 100 participant-years during the on-treatment period to 7.1 per 100 participant-years (8). In routine clinical settings, denosumab discontinuation (10) was associated with 4.7-fold higher risk for vertebral fracture than continuation. Risk also increased for major osteoporotic fracture (RR, 3.2 [CI, 2.2 to 4.8]) and hip fracture (RR, 5.3 [CI, 2.0 to 13.9]). In this study, we provide evidence that denosumab delay by more than 4 months is sufficient to cause a similar increase in vertebral fracture risk, whereas an increased risk for fracture at other anatomical sites cannot be ruled out. The risk difference between long delay and on-time doses might be underestimated in the current analysis. Estimates from sensitivity analyses excluding patients who had fractures in the 6 months before the start of follow-up showed that long delay led to higher risk for fracture than on-time administration for composite fracture (HR, 1.58 [CI, 1.04 to 2.41]), major osteoporotic fracture (HR, 2.00 [CI, 1.18 to 3.39]), and vertebral fracture (HR, 4.61 [CI, 1.78 to 11.9]). It might be inappropriate to conclude that risk was increased for all of these fractures, except for vertebral fracture, on the basis of these estimates. These results came from sensitivity analyses and could have multiple testing problems. However, they at least provide us a good motivation for larger studies, which can exclude or confirm such risks.

Without a placebo group, this study design cannot tell us whether the elevated fracture risk represents a reversal to the pretreatment baseline risk (that is, reduced therapeutic effect) or a rebound increase above the baseline (that is, true harm).

The major strength of this study is that we adopted a target trial emulation design, which helps align results from an observational study with those of actual randomized controlled trials (14, 39–43). Because the exposure of interest was time-related, we further used rigorous analysis methods to establish a correct, temporal, causal relationship between exposure and outcome, with exposure (that is, dosing delay) assigned before fracture events, providing a robust estimation of the effect of delay on fracture. This study had limited statistical power for composite fracture and several secondary end points (CIs were wide and included the null), except for vertebral fracture. Thus, evidence was insufficient to conclude that fracture risk was increased at other anatomical sites. Future studies with larger sample sizes are needed. Second, fracture diagnoses in primary care databases are underrecorded (44). Fracture incidence may be underestimated, especially for vertebral fractures. Such measurement bias would be nondifferential across study groups and would dilute an association, biasing the observed effect toward the null. Third, for each patient, we counted only the first fracture during follow-up, which may have led to further underestimation of the delay effect. On the basis of available evidence, patients who delay their subsequent injections are more likely to have multiple fractures, especially multiple vertebral fractures (8). We examined the occurrence of multiple vertebral fractures, but this end point could not be accurately determined in THIN. Future prospective studies with a well-adjudicated multiple vertebral fracture end point are needed. Fourth, we tried to examine the time course in increased fracture risk after delayed denosumab injection (Appendix Figure 4) and found an increase in vertebral fracture up to 24 weeks. However, whether this is the peak, plateau, or part of the decline remains unanswered. Fifth, the delay patterns were not randomly assigned and were thus susceptible to unmeasured

confounding. Such unmeasured confounding would be needed to have a combined effect size in excess of 7.28 to reduce the observed association between long delay and vertebral fracture to null. Last, reasons for delaying injections were not recorded in THIN; examining these reasons may help us develop strategies to improve adherence.

Although delayed administration of subsequent denosumab doses by more than 16 weeks is associated with increased risk for vertebral fracture compared with on-time administration, evidence is insufficient to conclude that risk for fracture is increased at other anatomical sites with long delay. Because patients who used denosumab were at high risk for vertebral fracture, strategies to improve timely administration of denosumab in routine clinical settings are needed.

Note: Drs. Solomon, Tang, and Lei had full access to the data, take responsibility for the content, and guarantee the integrity and accuracy of the work undertaken. The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Disclaimer: The interpretation of these data is the sole responsibility of the authors.

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Figure 1. Flow chart of eligible participants and denosumab doses, THIN 2010–2019. We compared the outcomes in 3 groups: “on time,” receiving the subsequent dose <4 wk after the

recommended date; “short delay,” receiving the subsequent dose 4–16 wk after the recommended date; and “long delay,” receiving the subsequent dose >16 wk after the recommended date. In clinical practice, patients who receive a subsequent dose “early” (i.e., 4 wk before the recommended date) are also considered to have received it on time. These injections were immediately censored after time 0 for the delay groups. THIN = The Health Improvement Network.

Figure 2. Cumulative risk for fractures. A. The composite fracture risk overlapped for on time, short delay, and long delay. Fracture risk increased in the long delay pattern after 20 wk of follow-up. Compared with on time, short delay had a fracture risk difference of 4.8/1000 (95% CI, −1.2/1000 to 10.1/1000) over 6 mo, with a hazard ratio (HR) of 1.03 (CI, 0.63 to 1.69); long delay had a fracture risk difference of 15.0/1000 (CI, 1.4/1000 to 33.5/1000) over 6 mo, with an HR of 1.44 (CI, 0.96 to 2.17). B. Compared with on time, short delay had a fracture risk difference of 1.4/1000 (CI, −0.6/1000 to 2.4/1000) over 6 mo, with an HR of 1.48 (CI, 0.58 to 3.79); long delay had a fracture risk difference of 7.9/1000 (CI, 1.1/1000 to 16.6/1000) over 6 mo, with an HR of 3.91 (CI, 1.62 to 9.45). C. Compared with on time, short delay had a fracture risk difference of 3.5/1000 (CI, −1.0/1000 to 6.7/1000) over 6 mo, with an HR of 0.94 (CI, 0.57 to 1.55); long delay had a fracture risk difference of 12.5/1000 (CI, 2.2/1000 to 26.1/1000) over 6 mo, with an HR of 1.69 (CI, 1.01 to 2.83). D. Compared with on time, short delay had a fracture risk difference of 0.2/1000 (CI, −2.9/1000 to 2.0/1000) over 6 mo, with an HR of 0.97 (CI, 0.44 to 2.12); long delay had a fracture risk difference of 3.7/1000 (CI, −2.3/1000 to 11.9/1000) over 6 mo, with an HR of 1.75 (CI, 0.81 to 3.79).

Appendix Figure 1. Study design of a hypothetical randomized controlled trial (“target trial”) on which we modeled our observational data analysis. We conceptualized a target trial comparing fracture risk between delays in subsequent dosing. Time 0 was set as the time when the subsequent injection should be administrated, with follow-up over the following 6 mo. We

compared the effect of 3 groups with different delays of the subsequent dose: “on time,” or within 4 wk after the recommended date; “short delay,” or 4–16 wk after the recommended date; and “long delay,” or >16 wk after the recommended date. This figure follows the recommendation on the visualization of study design from Schneeweiss and colleagues (18).

Appendix Figure 2. Target trial emulation with “cloning, censoring, and weighting” approach. To emulate the target trial in Appendix Figure 1 using observational data, we first created 3 copies of each eligible individual at baseline and assigned each of the replicates to each of the groups at time 0. Second, we censored replicates if and when they deviated from the assigned group, ensuring that replicates follow their assigned group. However, doing so may introduce selection bias, which can be eliminated by inverse probability of censoring weights in which uncensored observations are upweighted to represent censored observations with similar characteristics. The inverse probability of censoring weights 3 pseudopopulations. Differences in fracture events between the 3 pseudopopulations approximated the effect of the subsequent injections delay on fracture.

Appendix Figure 3. Cumulative risk for nonvertebral fracture. Compared with on time, short delay had a fracture risk difference of 2.9/1000 (95% CI, −3.0/1000 to 8.5/1000) over 6 mo, with a hazard ratio (HR) of 0.97 (CI, 0.57 to 1.66); long delay had a fracture risk difference of 8.6/1000 (CI, −4.1/1000 to 24.2/1000) over 6 mo, with an HR of 1.25 (CI, 0.80 to 1.94).

Appendix Figure 4. Association between delayed weeks and risk for fracture. Continuous dose–response relationship between delay and fracture risk. The *x*-axis represents subsequent dosing delay of 4–26 wk at weekly increments; the *y*-axis is the estimated hazard ratio of serial delay patterns with on-time dosing as the reference. Smoothed curves are used to show the association between weeks delayed and fracture risk.

Table 1. **Baseline Characteristics of Participants***

Characteristic	Study Population (*n* = 2594)
Mean age (SD), *y*	75.8 (9.5)
Women	2450 (94.4)
Mean BMI (SD), *kg/m²*	24.5 (5.3)
Smoking status	
Current	248 (9.6)
None	1625 (62.9)
Past	710 (27.5)
Cerebrovascular disease	75 (2.9)
Chronic pulmonary disease	148 (5.7)
Dementia	79 (3.0)
Diabetes	78 (3.0)
Peptic ulcer disease	9 (0.3)
Myocardial infarction	26 (1.0)
Peripheral vascular disease	21 (0.8)
Renal disease	121 (4.7)
Any cancer	60 (2.3)
Parkinson disease	19 (0.7)
Rheumatoid arthritis	36 (1.4)
Mean Charlson Comorbidity Index score (SD)	0.39 (0.80)
History of major osteoporotic fracture	1364 (52.6)
History of hip fracture	487 (18.8)
History of spine fracture	396 (15.3)
Mean 10-y risk for major osteoporotic fracture (SD), %†	22.0 (15.5)
Mean 10-y risk for hip fracture (SD), %†	18.7 (19.8)
Mean duration of oral bisphosphonate use (SD), *y*	3.1 (3.6)
Medication use	
Intravenous bisphosphonates	7 (0.3)
Hormone replacement therapy	50 (1.9)
SERMs	12 (0.5)
Teriparatide	4 (0.2)
Tricyclic antidepressant	382 (14.7)
Antiepilepsy drug	321 (12.4)
Systemic corticosteroids	538 (20.7)
Benzodiazepine	425 (16.4)
NSAIDs	1453 (56.0)
Opioids	820 (31.6)
PPI	1429 (55.1)
SSRI	372 (14.3)
Mean number of hospitalizations (SD)	1.8 (2.8)
Mean number of primary care visits (SD)	17.9 (15.1)
Mean number of referrals to the hospital (SD)	2.2 (2.3)

BMI = body mass index; NSAID = nonsteroidal anti-inflammatory drug; PPI = proton-pump inhibitor; SERM = selective estrogen receptor modulator; SSRI = selective serotonin reuptake inhibitor.

* Values are numbers (percentages) unless otherwise specified.
† Less than 1% of values were missing for BMI, race, and smoking history. To calculate the QFracture score, missing values were imputed by a sequential regression method based on predictors included in the QFracture 2012 algorithm (26).

Table 2. **Frequency, Rates, and Adjusted HRs of Fracture for On Time, Short Delay, and Long Delay Between Denosumab Doses***

Variable	On Time	Short Delay	Long Delay	*P* for Trend
Composite fractures†				
Weighted persons, *n*‡	8282	6771	6319	
Weighted events, *n*‡	243	208	269	
Risk over 6 mo (95% CI), *per 1000*	27.3 (19.1 to 36.2)	32.2 (24.1 to 43.5)	42.4 (27.7 to 64.6)	
Risk difference over 6 mo (95% CI), *per 1000*	Reference	4.8 (−1.2 to 10.1)	15.0 (1.4 to 33.5)	
Fully adjusted HR (95% CI)§	Reference	1.03 (0.63 to 1.69)	1.44 (0.96 to 2.17)	0.093
Sensitivity analysis HR (95% CI)‖	Reference	1.07 (0.63 to 1.82)	1.58 (1.04 to 2.41)	0.040
Major osteoporotic fractures†				
Weighted persons, *n*‡	8316	6813	6348	
Weighted events, *n*‡	144	108	184	
Risk over 6 mo (95% CI), *per 1000*	14.7 (9.6 to 18.6)	18.1 (12.7 to 21.9)	27.4 (17.4 to 39.2)	
Risk difference over 6 mo (95% CI), *per 1000*	Reference	3.5 (−1.0 to 6.7)	12.5 (2.2 to 26.1)	
Fully adjusted HR (95% CI)§	Reference	0.94 (0.57 to 1.55)	1.69 (1.01 to 2.83)	0.056
Sensitivity analysis HR (95% CI)‖	Reference	1.02 (0.60 to 1.73)	2.00 (1.18 to 3.39)	0.013
Vertebral fractures				
Weighted persons, *n*‡	8361	6984	6409	
Weighted events, *n*‡	21	25	62	
Risk over 6 mo (95% CI), *per 1000*	2.2 (1.1 to 3.6)	3.6 (1.6 to 5.4)	10.1 (3.5 to 19.1)	
Risk difference over 6 mo (95% CI), *per 1000*	Reference	1.4 (−0.6 to 2.4)	7.9 (1.1 to 16.6)	
Fully adjusted HR (95% CI)§	Reference	1.48 (0.58 to 3.79)	3.91 (1.62 to 9.45)	0.005
Sensitivity analysis HR (95% CI)‖	Reference	1.67 (0.60 to 4.62)	4.61 (1.78 to 11.9)	0.003
Hip fractures				
Weighted persons, *n*‡	8352	6934	6399	
Weighted events, *n*‡	43	33	58	
Risk over 6 mo (95% CI), *per 1000*	4.8 (2.7 to 7.1)	5.0 (2.4 to 6.9)	8.5 (3.3 to 16.1)	
Risk difference over 6 mo (95% CI), *per 1000*	Reference	0.2 (−2.9 to 2.0)	3.7 (−2.3 to 11.9)	
Fully adjusted HR (95% CI)§	Reference	0.97 (0.44 to 2.12)	1.75 (0.81 to 3.79)	0.173
Sensitivity analysis HR (95% CI)‖	Reference	1.02 (0.45 to 2.33)	1.91 (0.86 to 4.26)	0.132
Nonvertebral fractures				
Weighted persons, *n*‡	8286	6771	6329	
Weighted events, *n*‡	229	185	219	
Risk over 6 mo (95% CI), *per 1000*	25.8 (18.3 to 35.1)	28.7 (20.6 to 39.4)	34.4 (21.2 to 52.9)	
Risk difference over 6 mo (95% CI), *per 1000*	Reference	2.9 (−3.0 to 8.5)	8.6 (−4.1 to 24.2)	
Fully adjusted HR (95% CI)§	Reference	0.97 (0.57 to 1.66)	1.25 (0.80 to 1.94)	0.35
Sensitivity analysis HR (95% CI)‖	Reference	1.03 (0.58 to 1.81)	1.36 (0.86 to 2.15)	0.20

HR = hazard ratio.

* We compared the outcomes in 3 groups: “on time” indicates that the subsequent dose was received within 4 wk after the recommended date, “short delay” that the subsequent dose was received 4–16 wk after the recommended date, and “long delay” that the subsequent dose was received >16 wk after the recommended date.
† Composite fractures were fractures at any site. Major osteoporotic fractures included hip fracture, vertebral fracture, wrist fracture, humerus fracture, pelvis fracture, and rib fracture.
‡ Persons and events were calculated in the weighted population.
§ HRs were calculated from the final model, adjusting for baseline and postbaseline covariates; we used a robust SE to compute conservative 95% CIs for all of the HR estimates.
|| This model was the same as the primary analysis model, except that patients who had fractures in the 6 mo before the start of follow-up were excluded.

Table 3. **Subgroup Analyses, Stratified by Age, Prior Length of BP Use, and 10-Year Risk for Major Osteoporotic Fracture***

Variable	HR (95% CI) for Short Delay†	HR (95% CI) for Long Delay†	*P* for Trend	*P* for Interaction
Composite fractures				
Stratified by age				0.56
>75 y	1.14 (0.58–2.25)	1.58 (0.94–2.64)	0.091	
≤75 y	0.96 (0.52–1.80)	1.20 (0.59–2.43)	0.63	
Stratified by length of prior oral BP use				0.76
≤3 y	1.18 (0.58–2.38)	1.75 (1.01–3.03)	0.053	
>3 y	0.96 (0.54–1.70)	1.16 (0.61–2.21)	0.67	
Stratified by QFracture score‡				0.46
Fracture risk >20%	1.29 (0.66–2.52)	1.64 (0.92–2.90)	0.094	
Fracture risk ≤20%	0.72 (0.42–1.25)	1.22 (0.67–2.24)	0.57	
Major osteoporotic fractures				
Stratified by age				0.165
>75 y	0.84 (0.44–1.62)	2.11 (1.12–3.96)	0.028	
≤75 y	1.18 (0.53–2.61)	1.07 (0.44–2.63)	0.85	
Stratified by length of prior oral BP use				0.48
≤3 y	1.06 (0.52–2.16)	2.03 (0.99–4.14)	0.058	
>3 y	0.89 (0.44–1.80)	1.30 (0.60–2.82)	0.53	
Stratified by QFracture score‡				0.31
Fracture risk >20%	1.10 (0.52–2.35)	2.36 (1.13–4.95)	0.028	
Fracture risk ≤20%	0.79 (0.40–1.58)	1.21 (0.57–2.55)	0.66	
Vertebral fractures				0.55
Stratified by age				
>75 y	1.17 (0.27–5.09)	4.09 (1.17–14.3)	0.038	
≤75 y	2.07 (0.61–6.99)	3.39 (0.96–12.0)	0.082	
Stratified by length of prior oral BP use				0.31
≤3 y	1.49 (0.44–5.08)	4.83 (1.68–13.9)	<0.001	
>3 y	1.73 (0.39–7.63)	1.83 (0.33–10.2)	0.50	
Stratified by QFracture score‡				0.65
Fracture risk >20%	2.08 (0.44–9.73)	3.62 (0.75–17.4)	0.142	
Fracture risk ≤20%	1.21 (0.37–3.97)	4.52 (1.64–12.5)	0.008	
Hip fractures				
Stratified by age				0.51
>75 y	0.61 (0.21–1.79)	2.03 (0.76–5.45)	0.189	
≤75 y	1.82 (0.55–6.00)	1.51 (0.42–5.44)	0.47	
Stratified by length of prior oral BP use				0.76
≤3 y	1.57 (0.58–4.30)	2.31 (0.81–6.58)	0.127	
>3 y	0.51 (0.12–2.07)	1.51 (0.47–4.87)	0.55	
Stratified by QFracture score‡				0.064
Fracture risk >20%	1.66 (0.50–5.53)	4.08 (1.39–12.0)	0.015	
Fracture risk ≤20%	0.69 (0.24–2.01)	0.73 (0.22–2.43)	0.58	
Nonvertebral fractures				0.77
Stratified by age				
>75 y	1.10 (0.54–2.27)	1.32 (0.76–2.30)	0.33	

≤75 y	0.83 (0.42–1.65)	1.12 (0.52–2.41)	0.81	
Stratified by length of prior oral BP use				0.50
≤3 y	1.11 (0.52–2.36)	1.46 (0.80–2.66)	0.23	
>3 y	0.88 (0.47–1.63)	1.07 (0.54–2.12)	0.86	
Stratified by QFracture score‡				0.23
Fracture risk >20%	1.23 (0.60–2.51)	1.62 (0.90–2.91)	0.114	
Fracture risk ≤20%	0.65 (0.36–1.18)	0.83 (0.40–1.72)	0.57	

BP = bisphosphonate.

* We compared the outcomes in 3 groups: “on time” indicates that the subsequent dose was received within 4 wk after the recommended date, “short delay” that the subsequent dose was received 4–16 wk after the recommended date, and “long delay” that the subsequent dose was received >16 wk after the recommended date.
† The reference group was on-time dosing.
‡ Baseline 10-y risk for major osteoporotic fracture was evaluated with QFracture 2012 score (http://qfracture.org).

Appendix Table 1. **Specification and Emulation of a Target Trial of Denosumab Delay and Fracture Risk Using Observational Data**

Protocol Component	Target Pragmatic Trial Specification (A Hypothetical RCT That Is Ideal for Answering This Question)	Target Trial Emulation (Using Observational Data to Best Approximate the RCT Comparison)
Eligibility criteria	We set a 6-mo (180-d) "run-in" period to assess eligibility. Age ≥45 y between 2010 and 2019. Received a prior dose of denosumab 180 d before. Did not receive any other antiosteoporosis drug in the prior 180 d. ≥1 y of up-to-standard data in a THIN primary care practice.	Same as the target trial, except that patients could be eligible multiple times. Thus, we will emulate sequential trials and combine them. We will collect baseline covariates during the past 2 y.
Treatment strategies	1) On time: receive a subsequent dose of denosumab within 4 wk after randomization. 2) Short delay: receive a dose of denosumab 4–16 wk after randomization. 3) Long delay: receive a dose of denosumab >16 wk after randomization. In the 3 strategies, patients are not allowed to switch to any other antiosteoporosis drug (i.e., estrogens, selective estrogen receptor modulators, bisphosphonates, teriparatide, or combination of these medications).	Same as for the target trial. We define the date of denosumab injection using the date of denosumab prescription. In clinical practice, subsequent doses received early (i.e., 5–6 mo after the prior dose) are also viewed as on time. We will classify these injections as "on time" in the primary analysis and exclude them in the sensitivity analysis.
Treatment assignment	Eligible individuals are randomly assigned to 1 of the 3 "treatment strategies" and are aware of the strategy to which they have been assigned.	We classified patients according to the strategy that their data were comparable with at time 0 and emulated randomization by adjusting for baseline confounders.
Outcomes	Composite fracture (including all types of fracture), major osteoporotic fracture (hip, vertebral, wrist, humerus fracture, pelvis, and rib fracture), vertebral fracture, and hip fracture.	Same as for the target trial.
Follow-up	Starts at the time of assignment to a strategy and ends at the earliest of first fracture, death, 6 mo after time 0, or administrative end of follow-up.	Starts the day after the end of the "run-in" period.
Causal contrasts	ITT effect, per protocol effect.	Observational analogue of the per protocol effect.
Statistical analysis	ITT analysis: Per protocol effect (45): censor patients when they deviate from their assigned treatment strategy (not follow the predefined protocol, die, or switch/add other osteoporosis medications). The analysis will adjust for prerandomization and postrandomization prognostic factors that predict adherence to the protocol and loss to follow-up.	Observational analogue of the per protocol effect: same as target trial, except that we created 3 individuals (clones) per eligible person and assigned 1 to each treatment strategy. The analysis will adjust for baseline and postbaseline prognostic factors that predict adherence to the protocol and loss to follow-up.

ITT = intention-to-treat; RCT = randomized controlled trial; THIN = The Health Improvement Network.

Appendix Table 2. **Results of Sensitivity Analyses**

Variable	HR (95% CI) for Short Delay*	HR (95% CI) for Long Delay*	*P* for Linear Trend
Composite fractures			
Weighted model†	1.04 (0.64–1.71)	1.46 (0.96–2.20)	0.081
No fracture during run-in period‡	1.14 (0.65–1.97)	1.64 (1.06–2.53)	0.031
Additional IPW§	1.05 (0.63–1.76)	1.47 (0.96–2.24)	0.080
Delay <6 mo||	0.96 (0.64–1.45)	1.41 (0.85–2.33)	0.183
Effect of the second dose¶	0.67 (0.36–1.34)	1.45 (0.75–2.80)	0.30
Early injections excluded**	0.98 (0.51–1.88)	1.36 (0.82–2.26)	0.25
Major osteoporotic fractures			
Weighted model†	0.95 (0.57–1.58)	1.71 (1.02–2.87)	0.042
No fracture during run-in period‡	1.03 (0.61–1.76)	2.03 (1.18–3.50)	0.012
Additional IPW§	0.91 (0.55–1.56)	1.66 (0.97–2.83)	0.061
Delay <6 mo||	1.04 (0.61–1.76)	1.75 (0.96–3.18)	0.076
Effect of the second dose¶	0.96 (0.48–1.91)	2.45 (1.21–4.93)	0.019
Early injections excluded**	0.81 (0.43–1.52)	1.56 (0.82–2.98)	0.182
Vertebral fractures			
Weighted model†	1.49 (0.59–3.77)	3.92 (1.63–9.44)	0.007
No fracture during run-in period‡	1.70 (0.63–4.63)	4.73 (1.80–12.5)	0.005
Additional IPW§	1.45 (0.57–3.66)	3.95 (1.59– 9.86)	0.008
Delay <6 mo||	1.94 (0.72–5.22)	6.32 (2.40–16.6)	0.001
Effect of the second dose¶	1.08 (0.25–4.63)	4.05 (1.06–15.4)	0.057
Early injections excluded**	1.87 (0.64–5.44)	5.37 (1.89–15.3)	0.003
Hip fractures			
Weighted model†	1.01 (0.46–2.22)	1.85 (0.85–4.00)	0.134
No fracture during run-in period‡	1.03 (0.46–2.31)	2.00 (0.87–4.58)	0.116
Additional IPW§	0.97 (0.44–2.15)	1.78 (0.80–3.96)	0.169
Delay <6 mo||	1.17 (0.54–2.56)	2.09 (0.87–5.04)	0.12
Effect of the second dose¶	0.81 (0.26–2.49)	1.98 (0.62–6.33)	0.28
Early injections excluded**	0.87 (0.38–1.97)	1.72 (0.74–3.97)	0.22
Nonvertebral fractures			
Weighted model†	0.98 (0.57–1.68)	1.26 (0.81–1.97)	0.31
No fracture during run-in period‡	1.09 (0.60–1.97)	1.41 (0.88–2.25)	0.16
Additional IPW§	1.00 (0.57–1.74)	1.28 (0.81–2.02)	0.28
Delay <6 mo||	0.87 (0.56–1.34)	1.08 (0.62–1.90)	0.77
Effect of the second dose¶	0.64 (0.33–1.23)	1.20 (0.58–2.48)	0.67
Early injections excluded**	0.90 (0.45–1.82)	1.13 (0.66–1.95)	0.65

HR = hazard ratio; IPW = inverse probability weight.

* The reference group was on-time dosing.
† We used inverse probability treatment weights instead of multivariate regression to control potential baseline confounding.
‡ A sensitivity analysis that excluded patients who had fractures during the run-in period.
§ A sensitivity analysis that used additional inverse probability of censoring weights to address potential bias due to attrition of later doses. In our study population, only a proportion of the 2594 patients received the third dose (79%) and fourth dose (59%). Because of the concern of potential selection bias of patients attrition in the later emulated trials, we used an additional IPW to address potential bias.
|| A sensitivity analysis done in a subset population who received their subsequent injection in 6 mo.
¶ A sensitivity analysis that examined the relationship between delay and fracture risk of the second dose only.
** A sensitivity analysis that excluded patients who had “early” injections before the start of follow-up.

Appendix Table 3. **Examining the Effect of Unmeasured Confounding With E-values**

Variable	Long Delay*	E-Value Estimate†	E-Value Lower Limit of the CI
Composite fractures			
HR (95% CI)	1.44 (0.96 to 2.17)	2.23	1.00
Sensitivity analysis HR (95% CI)	1.58 (1.04 to 2.41)	2.54	1.24
Major osteoporotic fractures			
HR (95% CI)	1.69 (1.01 to 2.83)	2.77	1.11
Sensitivity analysis HR (95% CI)	2.00 (1.18 to 3.39)	3.41	1.64
Vertebral fractures			
HR (95% CI)	3.91 (1.62 to 9.45)	7.28	2.62
Sensitivity analysis HR (95% CI)	4.61 (1.78 to 11.9)	8.69	2.96
Hip fractures			
HR (95% CI)	1.75 (0.81 to 3.79)	2.90	1.00
Sensitivity analysis HR (95% CI)	1.91 (0.86 to 4.26)	3.23	1.00
Nonvertebral fractures			
HR (95% CI)	1.25 (0.80 to 1.94)	1.81	1.00
Sensitivity analysis HR (95% CI)	1.36 (0.86 to 2.15)	2.06	1.00

CI = confidence interval; HR = hazard ratio.

* The reference group was on-time dosing.
† The E-value is defined as the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have to fully explain away a specific treatment–outcome association, conditional on the measured covariates. A large E-value indicates that considerable unmeasured confounding would be needed to explain away an effect estimate (31). We found an E-value of 7.28 with a lower confidence limit of 2.62 for vertebral fracture, meaning that a substantial unmeasured confounding is needed to explain away the association between long delay and vertebral fracture risk to null. The E-value for composite fracture was 2.23, with a lower confidence limit of 1.00. This suggests a more robust causal relationship between long delay and vertebral fracture than between long delay and composite fracture.

Figure 1

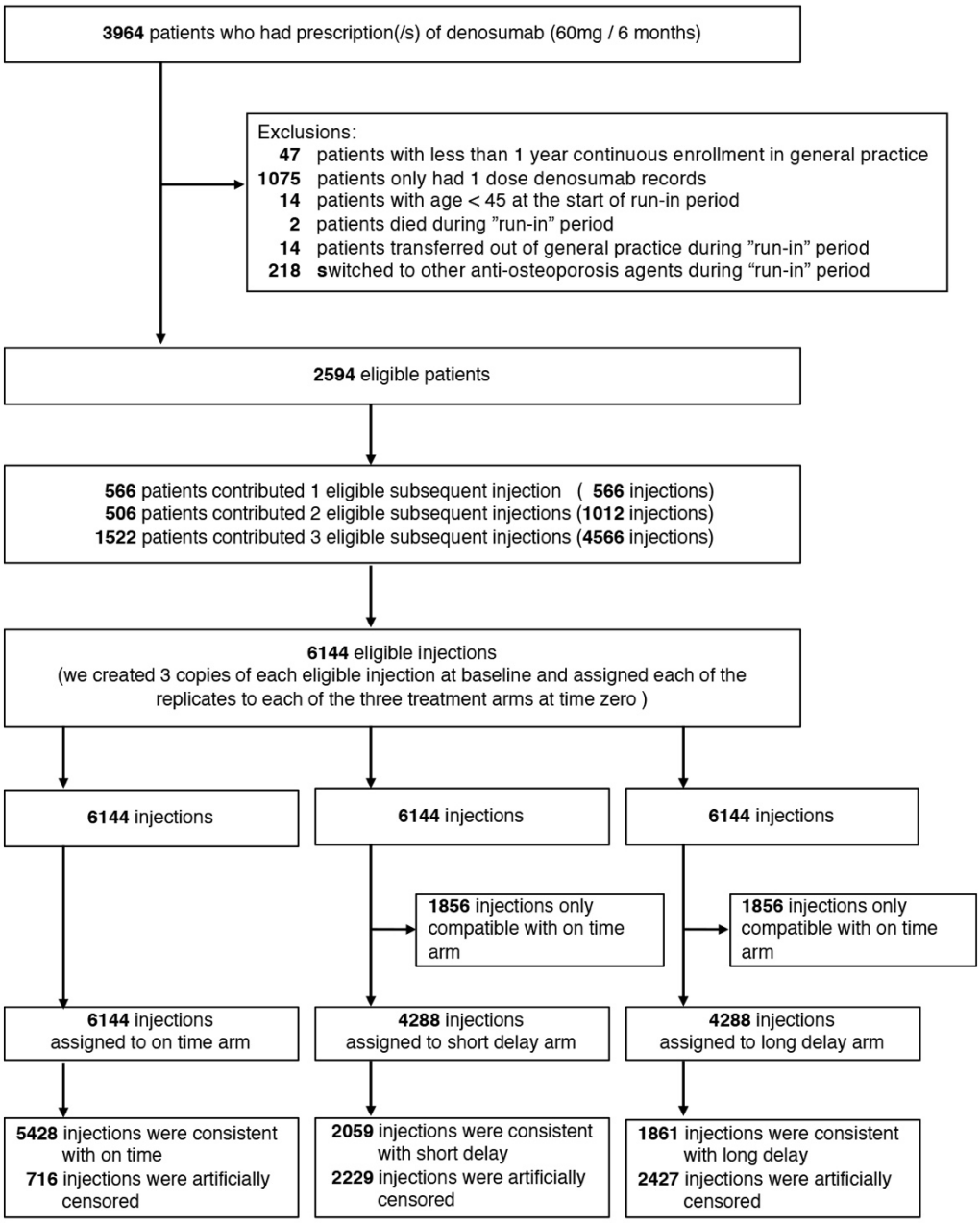
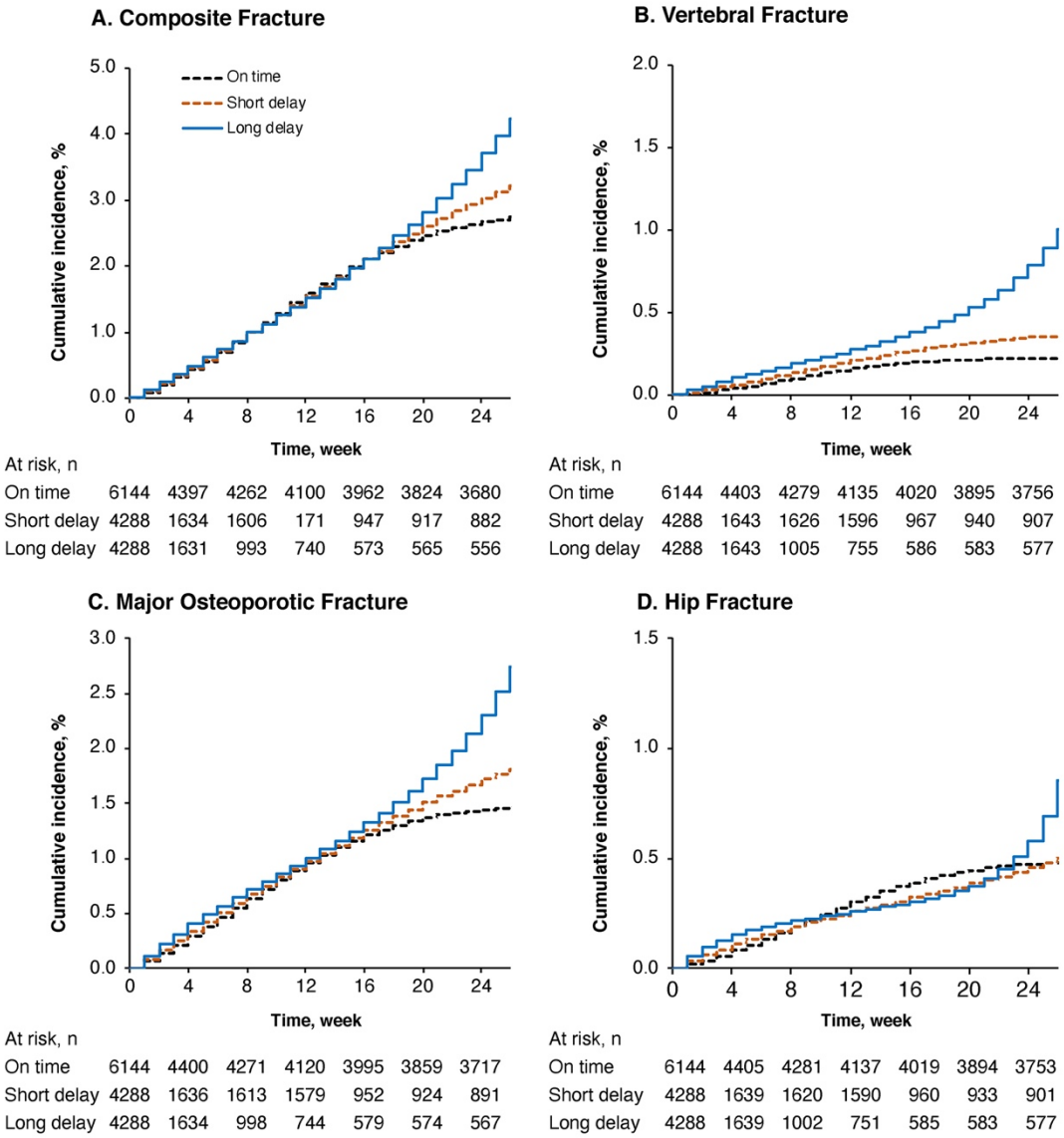
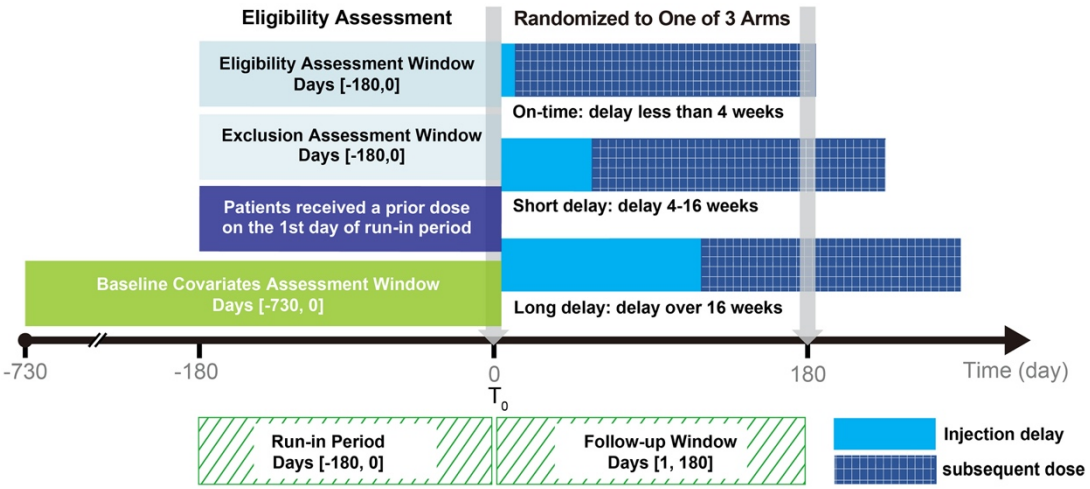

3964 patients who had prescription(/s) of denosumab (60mg / 6 months)
Exclusions:
47 patients with less than 1 year continuous enrollment in general practice
1075 patients only had 1 dose denosumab records
14 patients with age < 45 at the start of run-in period
2 patients died during "run-in" period
14 patients transferred out of general practice during "run-in" period
218 switched to other anti-osteoporosis agents during "run-in" period
2594 eligible patients
566 patients contributed 1 eligible subsequent injection (566 injections)
506 patients contributed 2 eligible subsequent injections (1012 injections)
1522 patients contributed 3 eligible subsequent injections (4566 injections)
6144 eligible injections
(we created 3 copies of each eligible injection at baseline and assigned each of the replicates to each of the three treatment arms at time zero)
6144 injections
6144 injections
6144 injections
1856 injections only compatible with on time arm
1856 injections only compatible with on time arm
6144 injections assigned to on time arm
4288 injections assigned to short delay arm
4288 injections assigned to long delay arm
5428 injections were consistent with on time
716 injections were artificially censored
2059 injections were consistent with short delay
2229 injections were artificially censored
1861 injections were consistent with long delay
2427 injections were artificially censored

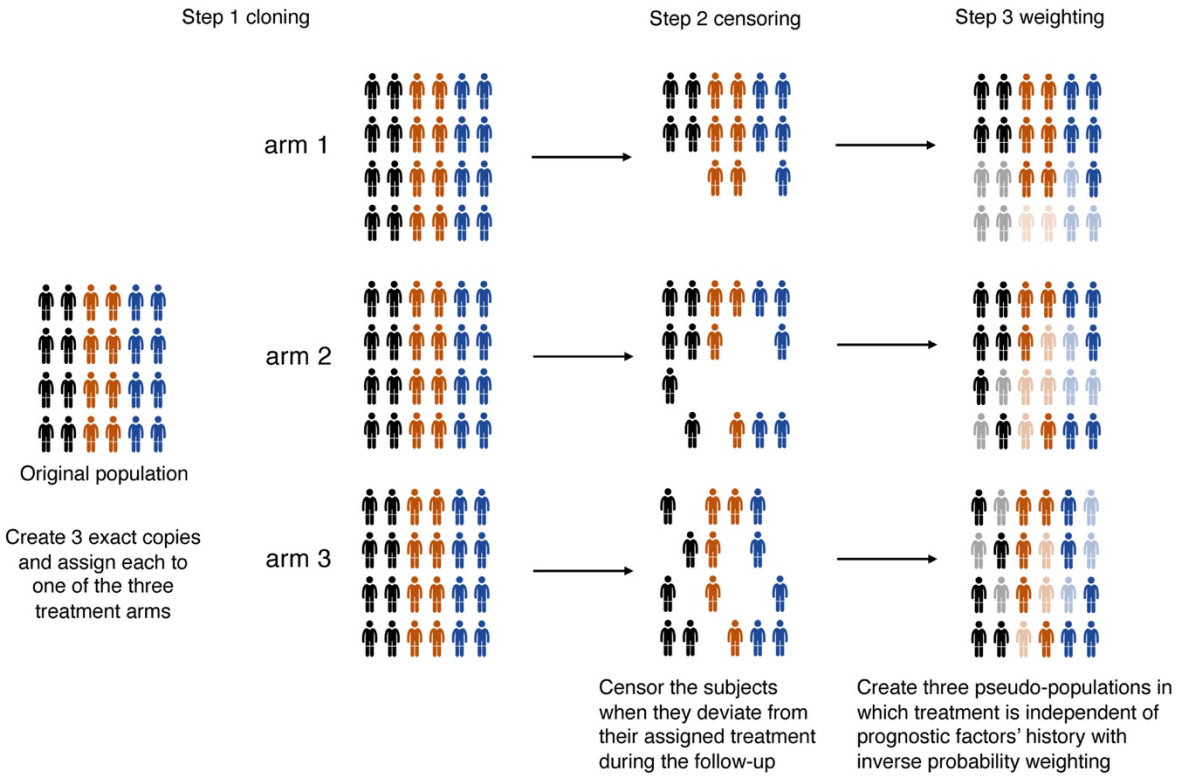
Figure 2


A. Composite Fracture
Cumulative incidence, %
On time
Short delay
Long delay
Time, week
At risk, n
On time 6144 4397 4262 4100 3962 3824 3680
Short delay 4288 1634 1606 171 947 917 882
Long delay 4288 1631 993 740 573 565 556
B. Vertebral Fracture
Cumulative incidence, %
Time, week
At risk, n
On time 6144 4403 4279 4135 4020 3895 3756
Short delay 4288 1643 1626 1596 967 940 907
Long delay 4288 1643 1005 755 586 583 577
C. Major Osteoporotic Fracture
Cumulative incidence, %
Time, week
At risk, n
On time 6144 4400 4271 4120 3995 3859 3717
Short delay 4288 1636 1613 1579 952 924 891
Long delay 4288 1634 998 744 579 574 567
D. Hip Fracture
Cumulative incidence, %
Time, week
At risk, n
On time 6144 4405 4281 4137 4019 3894 3753
Short delay 4288 1639 1620 1590 960 933 901
Long delay 4288 1639 1002 751 585 583 577

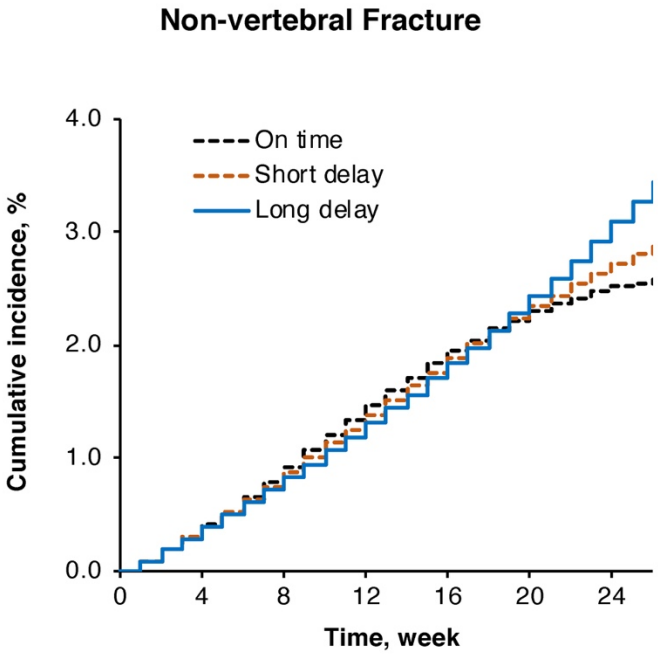
Supplement figure 1



Supplement figure 2



Supplement figure 3



At risk, n							
On time	6142	4398	4263	4104	3967	3832	3688
Short delay	4288	1635	1608	1574	950	920	885
Long delay	4288	1632	993	740	573	565	557

Supplement figure 4