Denosumab for Prevention of Post-Teriparatide Bone Loss in Premenopausal Women with Idiopathic Osteoporosis: a Phase IIB Study

NCT02049866

Date: 1/17/2017

Denosumab for Prevention of Post-Teriparatide Bone Loss in Premenopausal Women with Idiopathic Osteoporosis: a Phase IIB Study

FD-R-05114-01
IRB AAAN0161
Protocol Version 3.0 Revised 1/17/2017

1. Study Purpose and Rationale

Osteoporosis in premenopausal women with normal menstrual function and no specific cause is termed idiopathic osteoporosis (IOP). IOP is a rare disease with an estimated prevalence of <200,000 affected premenopausal women in the United States. We recently completed an NIH-funded study of 64 premenopausal women with IOP, most with major osteoporotic fractures[1]. High resolution peripheral quantitative computed tomography (HR-pQCT), central QCT, and microCT of iliac bone biopsies documented that, compared to 40 normal controls, women with IOP have substantial microstructural deficits: thinner more porous cortices; fewer, thinner, more widely separated and heterogeneously distributed trabeculae; and markedly lower bone stiffness[2-4]. To determine whether teriparatide (TPTD), an osteoanabolic drug that increases bone formation, improved bone microarchitecture and strength in premenopausal women with IOP, we conducted an open-label, 24-month pilot study of TPTD, 20 µg daily, in 21 affected women. There were impressive gains in areal BMD (aBMD) by dual energy x-ray absorptiometry (DXA) at the spine (10.8 ± 6.4%), total hip (6.2 ± 5.7%) and femoral neck (7.6 ± 3.4%), and no significant change at the forearm[5]. Paired transiliac bone biopsies before and after TPTD revealed improved trabecular structure and a 71% increase in trabecular stiffness. Cortical thickness increased by 22% but cortical porosity also increased by 46%[5]. By HR-pQCT, trabecular structure and whole bone stiffness improved, despite a 17% increase in cortical porosity at the radius[6].

Most patients require antiresorptive therapy to maintain TPTD-induced increases in BMD. However, several studies have shown that BMD remains stable after TPTD is discontinued in postmenopausal women on estrogen[7-9] and in premenopausal women who regained normal menses after completing a course of nafarelin for endometriosis[10]. Based on these studies, we hypothesized that the gains in BMD would be maintained in the IOP-TPTD pilot study participants because they were menstruating and estrogen replete. Therefore, we did not institute sequential antiresorptive therapy. However, contrary to our hypothesis, recent analyses of follow-up BMDs revealed that participants in the pilot study sustained average losses of 5.1 ± 4.3% at the spine and 1.4 ± 4.4% at the femoral neck over the 1-2 years after TPTD discontinuation.

We are currently enrolling 41 premenopausal women with IOP into a new, randomized, 24-month, FDA Orphan Diseases Program-funded trial, “A Phase 2 Study of Teriparatide for the Treatment of Idiopathic Osteoporosis in Premenopausal Women” (FD003902; PI, Shane). The follow-up data from our pilot study lead us to conclude that participants in FD003902 will require antiresorptive treatment to prevent bone loss after completing TPTD. Denosumab, a potent inhibitor of osteoclast-mediated bone resorption, leads to continuous gains in both trabecular and cortical BMD. Moreover, denosumab is not retained in the skeleton, and may thus be preferable for use in young women who may be contemplating future pregnancies. We hypothesize that denosumab, initiated after completion of two years of TPTD, will maintain or improve central and peripheral areal and volumetric BMD, microstructure and stiffness in premenopausal women with IOP. We will test this hypothesis in a 24-month study of denosumab (Prolia®, 60mg SC every 6 months). As planned enrollment in FD003902 is 41 women and some may not choose to participate, power is too low for a randomized, placebo-controlled design. Therefore, we plan an open-label, pilot study. The goals of the study are to estimate the effects of denosumab on central and peripheral, as well as trabecular and cortical, bone mass and microstructure and to obtain preliminary data to inform the design of a future randomized study. This study presents the first opportunity to study the effects of denosumab after TPTD in this unique and severely affected group of young women.

2. Study Design and Statistical Procedures

This study, entitled "Denosumab for the prevention of post-teriparatide bone loss in premenopausal women with idiopathic osteoporosis", is an observational prospective, non-randomized, open-label, pilot study of denosumab for prevention of bone loss after completing a 24-month course of TPTD in premenopausal women with IOP. The study will enroll women who have completed at least 12 months of Forteo treatment for the FDA Orphan Disease Branch-funded study, "Phase 2 Study of Teriparatide (TPTD) for the Treatment of Idiopathic Osteoporosis in Premenopausal Women (FD003902; PI Shane)." Thus, inclusion and exclusion criteria and sample size estimates rely on the original design of FD003902. Denosumab (Prolia®, 60mg) will be administered to participants in FD003902 every 6 months for 6 doses by subcutaneous injection according to standard procedures. The study will involve a minimum of one screening visit and a baseline visit, which will be completed during their participation in FD003902, and another 7 study visits over 36 months. The final measurements in FD003902 are the same as the baseline Denosumab measurements. Areal BMD by DXA will be measured at baseline (completion of FD003902), and at the 12 month, 24 month and 36 month visits after initiating denosumab. Volumetric BMD (vBMD) of the lumbar spine by cQCT will be performed at baseline and after 12 months of denosumab and of the distal radius and tibia by HR-pQCT will be performed at baseline and after 12, 24 and 36 months of denosumab (Table 2). Participants may choose to decline study drug and still complete yearly observational follow up visits thus enrolling in the control study arm.

Statistical Analysis Plan

2.A. Statistical Analysis Plan

The Specific Aims of this study are as follows:

Aim 1: To estimate the effect of 12, 24 and 36 months of denosumab on areal BMD of the lumbar spine, total hip, femoral neck and one-third radius. The primary outcome will be change in lumbar spine areal BMD by DXA at 12 months.

Aim 2: To estimate the effect of 12 months of denosumab on total and trabecular volumetric BMD and stiffness of the lumbar spine assessed by central QCT of L1/L2 and finite element analysis of central QCT datasets.

Aim 3: To estimate the effect of 12, 24 and 36 months of denosumab on total, cortical, and trabecular volumetric BMD, trabecular plate and rod microarchitecture, cortical porosity and stiffness of the distal radius and tibia by HR-pQCT and finite element analysis of HR-pQCT datasets.

Aim 4: To estimate the effect of 12, 24 and 36 months of denosumab on serum PTH and bone turnover markers (CTX, P1NP, osteocalcin) and to assess associations between these measurements and the effects of denosumab on areal and volumetric BMD, microarchitecture and stiffness.

Hypothesis 1: We hypothesize that, in premenopausal women with IOP who have completed 24 months of TPTD, treatment with denosumab will lead to additional gains in areal BMD by DXA at the spine, total hip, and femoral neck, and also to increases at the distal radius not seen during TPTD treatment.

Expected Results: Although there are no published data on the effects of denosumab on BMD in premenopausal women or in patients after completing a course of TPTD, several studies have documented gains in areal BMD at trabecular sites over 24 months of denosumab treatment in postmenopausal women[27,28,30] and in men[11]. In addition, several studies have shown that areal BMD by DXA continues to increase at the spine and hip, by an additional 4-5%, in patients who take alendronate after completing a course of PTH[12-14]. In addition, Leder et al. recently reported that postmenopausal women who completed 24 months of TPTD followed by 24 months of denosumab, experienced additional significant improvements in BMD at the spine (+6.2%), total hip (3.8%) and femoral neck (+4.0%)[15]. We therefore expect that areal BMD by DXA will increase to a similar extent (by about 4-5%) in the premenopausal women with IOP who take denosumab after completing FD003902. Moreover, as improvements at the predominantly cortical one-third

radius have also been reported in patients receiving denosumab[11,16], we expect similar increases of 0.6-1.4% at this site. We expect that the direction and extent of changes in areal BMD in FD003902 completers who take denosumab will differ significantly from FD003902 completers who decline denosumab in whom we expect BMD to decrease, and also from women in the pilot study, in whom mean BMD decreased by 5.1 ± 4.3% at the spine, 1.4 ± 4.4% at the femoral neck, 1.3 ± 3.4% at the total hip, with no change at the one-third radius (+0.1 ± 2.5%). Our power analyses estimate that, given the expected sample size of 30 FD003902 completers who take denosumab, we would have 80% power (5% alpha) to detect BMD changes of 2.24% or more. Based on the changes expected above, we expect to be able to detect significant changes in LS aBMD at 12 months (our primary outcome) as well as significant changes at other aBMD sites.

Hypothesis 2: We hypothesize that, in premenopausal women with IOP who have completed a 24 month course of TPTD, treatment with denosumab will lead to further increases in total and trabecular volumetric BMD (vBMD) and stiffness of the lumbar spine assessed by cQCT of L1/L2 and FEA of cQCT datasets.

Expected Results: In a subset of over 60 postmenopausal women in the FREEDOM trial, volumetric BMD by cQCT was measured at the spine and hip at baseline, 12, 24 and 36 months[17-19]. By 36 months, volumetric BMD increased in those who received denosumab by 6.4% ($p<0.0001$) at the total hip, and by 9.9% ($p<0.0001$) at the trabecular compartment[17]. At the lumbar spine, increases in total volumetric BMD were larger, with a reported 21.8% ($p<0.0001$) difference between the denosumab and placebo groups[19]. FEA of cQCT datasets from the FREEDOM subset showed an 18.2% increase in spine strength ($p<0.0001$) at 36 months[18]. In FD003902 completers, we expect to observe large increases in total volumetric BMD and strength of the lumbar spine after TPTD treatment, as has been observed in other cQCT studies of patients treated with TPTD[20,21]. In FD003902 completers who then take denosumab, we expect at a minimum, that lumbar spine volumetric BMD will remain stable, as was observed when alendronate followed PTH(1-84) in the PaTH study[12]. It is also possible that we may observe further increases in lumbar spine volumetric BMD; this could occur either because suppression of resorption by denosumab in the largely trabecular vertebral body permits mineralization of newly deposited trabecular bone or because denosumab could improve microarchitecture of the thin cortical rim of the vertebral body. In contrast, we expect significant losses in FD003902 completers who decline denosumab, comparable to the 10% decline at the spine observed in postmenopausal women in the PaTH study who were randomized to placebo after PTH(1-84)[12]. We will not be able to compare FD003902 completers who take denosumab to women from the pilot study, because volumetric BMD measured by central QCT was not performed in that study. Our power analyses estimate that, given the expected sample size of 30 FD003902 completers who take denosumab, we will have 80% power (5% alpha) to detect within-group changes in lumbar spine trabecular volumetric BMD of 5.61% or more. In addition, given the expected sample size of 30 FD003902 completers who take and 8 who decline denosumab, we will have 80% power (5% alpha) to detect between-group differences in change in lumbar spine trabecular volumetric BMD of 11.85%.

Hypothesis 3: We hypothesize that, in premenopausal women with IOP who have completed a 24 month course of TPTD, treatment with denosumab will lead to increases in total, cortical and trabecular vBMD, improvement in trabecular plate and rod microarchitecture, decreases in cortical porosity and increases in whole bone stiffness of the distal radius and tibia by HR-pQCT and FEA of HR-pQCT datasets.

Expected Results: A concern that was raised by our pilot study of TPTD in premenopausal women with IOP were the increases in cortical porosity observed at the iliac crest[5] and the distal radius[6] by HR-pQCT. Although whole bone stiffness increased at the radius despite the increase in cortical porosity, the improvement might have been larger had cortical porosity not increased. In contrast to TPTD, denosumab has been associated with improvements in cortical microarchitecture and porosity. In a 12-month study of 247 postmenopausal women randomized to placebo, alendronate or denosumab, HR-pQCT studies revealed significant increases in total, cortical and trabecular volumetric BMD and cortical thickness at both the distal radius and tibia in response to denosumab[22]. Total, trabecular and cortical vBMD increased by 0.5-2.5% and cortical thickness increased by 3-5% at 12 months in the denosumab treated subjects. These changes were significantly different than those seen in the placebo group, which had declines in these same measures[22]. Histomorphometric studies in ovariectomized cynomolgus monkeys treated with denosumab show marked reductions in both trabecular eroded surface and cortical porosity in response to this potent antiresorptive medication[23]. We

expect to detect similar changes by HR-pQCT in our premenopausal IOP FD003902 completers, who then take denosumab, while cortical and trabecular parameters may remain stable or deteriorate further in those who decline to take denosumab, as we observed in the women in the pilot study.

Many of our advanced analysis techniques, including individual trabecular segmentation, cortical porosity analyses, and FEA models scaled for mineralization, have not been applied to data obtained in subjects treated with denosumab. This study will provide an opportunity to use these advanced methodologies to examine the effect of denosumab on microarchitecture and stiffness in novel ways.

Hypothesis 4: We hypothesize that, in premenopausal women with IOP who have completed a 24 month course of TPTD, treatment with denosumab will lead to increased serum PTH levels and suppression of serum bone remodeling markers.
Expected Results: Studies of postmenopausal women treated with denosumab have documented sustained decreases of >60% in P1NP, >40% in Osteocalcin and >80% in CTX[22,24-27] lasting over many years of treatment[25]. Similar decreases in CTX of about 80% have also been documented in men with osteoporosis treated with denosumab[11]. Thus we expect similar changes in CTX, Osteocalcin and P1NP in this study. In postmenopausal women with osteoporosis, serum intact PTH levels have been reported to increase in a dose-related manner by one month after denosumab initiation and to return to baseline by 6 months after injection[24]. This rise in PTH has been associated with declines in cortical porosity assessed by HR-pQCT[28], a paradoxical response as, in general, PTH is catabolic for cortical bone and excess PTH secretion is associated with increases in cortical porosity. The paradoxical relationship between serum PTH and cortical porosity with denosumab is thought to be because PTH requires RANK Ligand for its catabolic actions at cortical bone and denosumab specifically blocks RANK Ligand, shifting the pathway of endogenous PTH action from the catabolic effects usually seen at cortical bone to an anabolic pathway usually seen at trabecular bone. Therefore, we expect to see improvements in trabecular and cortical volumetric BMD and declines in cortical porosity with denosumab that are directly related to the increases in PTH and decreases in CTX and P1NP.

ANALYSIS PLAN

Sample Size Considerations

Planned enrollment for FD003902 is 41 women. After our pilot study of 21 women, we succeeded in obtaining follow-up data post-TPTD treatment in 76%, but this was not a preplanned follow-up. In FD003902, all participants have already consented to one year of post-TPTD follow-up. However, it is possible that some will not enroll in the denosumab pilot extension study. **We conservatively estimate that 30 of the 41 women will agree to enroll in the denosumab extension study and that another 8 women will agree to be followed without intervention**. Because of these sample size limitations, this pilot study is planned as an open label investigation, with goals to estimate the effect of denosumab on central and peripheral, as well as trabecular and cortical, bone mass and microstructure and to obtain preliminary data to inform the design of a future randomized study.

Primary and Secondary Outcome Variables

Specific Aims: In premenopausal women with IOP who have completed 24 months of TPTD, to estimate the effect of 12**, 24 and 36** months of denosumab on:
Aim 1: Areal BMD by DXA of the lumbar spine, total hip, femoral neck and one-third radius

Primary outcome variable: within-group difference (percent change) in BMD at the

- Lumbar spine (L1-4) between the 24-month TPTD visit (baseline) and the 12-month denosumab visit

Secondary outcome variables: within-group difference (percent change) in BMD by DXA at the

- Lumbar spine (L1-4) between the 24-month TPTD visit and the 24-month and 36-month denosumab visits
- Total hip, femoral neck and 1/3rd radius aBMD between the 24-month TPTD visit and the 12-month and 24-month and 36-month denosumab visits

Aim 2: Total and trabecular volumetric BMD and stiffness of the lumbar spine assessed by central QCT and finite element analysis of central QCT datasets after 12 months of denosumab

Primary outcome variable: the within-group difference (percent change) between the 24-month TPTD visit and the 12-month denosumab visit in

- L1/2 trabecular vBMD by cQCT

Secondary outcome variables: the within-group change (percent change) in

- L1/2 trabecular vBMD by cQCT between the 24-month TPTD visit and 12-month denosumab visit
- L1/2 stiffness by FEA of cQCT scans between the 24-month TPTD visit and 12 -month denosumab visit

Aim 3: Total, cortical and trabecular volumetric BMD, trabecular plate and rod microarchitecture, cortical porosity and stiffness of the distal radius and tibia by HR-pQCT and finite element analysis of HR-pQCT datasets

Primary outcome variable: the within-group difference (percent change) between the 24-month TPTD visit and the 12-month denosumab visit in

- Trabecular volumetric density of the distal radius and tibia

Secondary outcome variables: the within-group difference (percent change) in

- Trabecular volumetric density of the distal radius and tibia between the 24-month TPTD visit and the 24-month and 36-month denosumab visits

and the within-group difference (percent change) between the 24-month TPTD visit and the 12 and 24- and 36-month denosumab visits in

- Total volumetric density and trabecular number of the distal radius and tibia by HR-pQCT
- Trabecular plate and rod number by ITS of HR-pQCT datasets
- Cortical density, thickness, and porosity of the distal radius and tibia by HR-pQCT
- Whole bone stiffness of the distal radius and tibia by FEA of HR-pQCT scans

Aim 4: Serum PTH and bone turnover markers (C-telopeptide, Osteocalcin, Procollagen type 1 N-terminal propeptide (P1NP)) and the associations between these variables and the effects of denosumab on areal and volumetric BMD, microarchitecture and stiffness

Primary outcome variables: the within-group difference (percent change from 24-month TPTD visit) and net Area Under the Curve (AUC) during the first 12 months of denosumab in

- Serum C-telopeptide, osteocalcin, P1NP

Secondary outcome variables:

- Within-group difference (percent change from 24-month TPTD visit) and net AUC during 12 months of denosumab in serum PTH
- Associations between net AUC of serum C-telopeptide, osteocalcin and P1NP during first 12 months of denosumab and 12 month percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
- Associations between net AUC of serum C-telopeptide, osteocalcin and P1NP during first 12 months of denosumab and 24 month percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
    - Total, cortical and trabecular volumetric BMD and whole bone stiffness of radius and tibia by HR-pQCT and FEA of HR-pQCT datasets
- Associations between net AUC of serum PTH during first 12 months of denosumab and 12 month percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
- Associations between net AUC of serum PTH during first 12 months of denosumab and 24 month

percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
    - Trabecular volumetric BMD and stiffness of L1/2 by cQCT and FEA of cQCT datasets
    - Total, cortical and trabecular volumetric BMD and whole bone stiffness of radius and tibia by HR-pQCT and FEA of HR-pQCT datasets
- Associations between net AUC of serum C-telopeptide, osteocalcin and P1NP during first 12 months of denosumab and 36 month percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
    - Total, cortical and trabecular volumetric BMD and whole bone stiffness of radius and tibia by HR-pQCT and FEA of HR-pQCT datasets
- Associations between net AUC of serum PTH during first 12 months of denosumab and 36 month percent change in
    - Areal BMD by DXA of the lumbar spine, total hip, femoral neck and 1/3 radius
    - Total, cortical and trabecular volumetric BMD and whole bone stiffness of radius and tibia by HR-pQCT and FEA of HR-pQCT datasets

We will undertake 3 sets of analyses, the main analysis and two exploratory analyses:
Analysis 1, the main analysis, is the denosumab within-group analysis.
In addition to the main analysis, two sets of exploratory analysis (Analyses 2 and 3) will be performed to assess whether there are differences between women completing TPTD who are denosumab-treated and untreated.
Analysis 2 is a comparison of the prospectively treated denosumab group to the prospectively followed, but untreated, concurrent control group.
Analysis 3 is a comparison of the prospectively treated denosumab group to the 14 historical subjects from the pilot study (see Sections 3C.6 and 3C.7) with follow-up data after the cessation of TPTD.

POWER ANALYSIS
For **Analysis 1** (the main denosumab within-group analysis), to estimate the confidence interval for an absolute change in bone structure and turnover following denosumab, the power calculation proceeds from the assumption of a two-tailed one-sample T-test against a constant of 0 (zero) change and uses the variances observed in our historical cohort. Given a sample size of 30 subjects who agree to take denosumab, we expect to able to detect, at a minimum, the following differences at 12 or 24 or 36 months:
Lumbar spine aBMD by DXA: difference of 2.24% when the SD is 4.30%,
Total hip aBMD by DXA: difference of 2.00% when the SD is 3.84%,
One-third radius aBMD by DXA: difference of 1.26% when the SD is 2.43%,
L1/2 Trabecular (Tb) vBMD by cQCT: difference of 5.61% when the SD is 10.78%
Radius trabecular vBMD by HR-pQCT: difference of 0.84% when the SD is 1.61%
Tibia cortical volumetric BMD (Ct. vBMD) by HR-pQCT: difference of 1.11% when the SD is 2.14%.
Serum CTX: difference of 5.2% when the SD is 10%.
Serum PINP: difference of 10.4% when the SD is 20%.

For **Analysis 2**, we will estimate the difference at 12 months in the change from cessation of TPTD in the prospectively treated denosumab group to the change observed in the prospectively followed, but untreated, concurrent control group, again using a two-tailed independent T-test. We assume that at least 8 of the 10 subjects who chose not to enter the denosumab open-label study will continue participation (observation) and provide data (the concurrent control group analysis). Given this assumed sample size of 30 treated subjects, compared to 8 untreated concurrent controls, we expect to be able to detect, at a minimum, the following differences:
Lumbar spine aBMD by DXA: difference of 4.92% when the SD is 4.30%
Total hip aBMD by DXA: difference of 4.39% when the SD is 3.84%
One-third radius aBMD by DXA: difference of 2.78% when the SD is 2.43%
Radius trabecular vBMD by HR-pQCT: difference of 1.84% when the SD is 1.61%
Tibia cortical volumetric BMD (Ct. vBMD) by HR-pQCT: difference of 2.45% when the SD is 2.14%.

For **Analysis 3**, we will estimate the 12-month difference in the change from cessation of TPTD in the prospectively treated denosumab group against the data available from the 14 historical subjects from the pilot study followed after the cessation of TPTD (see Sections 3C.5 and 3C.6), using a two-tailed independent T-test. Given this assumed sample size of 30 newly treated subjects and 14 historical controls, we expect to be able to detect, at a minimum, the following differences:
Lumbar spine aBMD by DXA: 3.91% when the SD is 4.30%
Total hip aBMD by DXA: difference of 3.49% when the SD is 3.84%
Radius trabecular vBMD by HR-pQCT: difference of 1.46% when the SD is 1.61%
Tibia cortical volumetric BMD (Ct. vBMD) by HR-pQCT: difference of 1.96% when the SD is 2.14%.

The differences detectable assuming 80% power and 5% alpha with fixed sample sizes and the analyses described are shown as differences in percentage points, calculated from our preliminary studies, in Table 1.

Table 1	Analysis 1 Main Analysis	Analysis 2	Analysis 3
	Within Dmab Δ	vs. Concurrent Δ	vs. Historical Δ
DXA LS BMD	2.24%	4.92%	3.91%
DXA TH BMD	2.00%	4.39%	3.49%
DXA one-third radius BMD	1.26%	2.78%	2.21%
cQCT lumbar spine Tb vBMD	5.61%	11.85%	N/A
HR-pQCT Radial Tb vBMD	0.84%	1.84%	1.46%
HR-pQCT Radial Ct Thickness	2.86%	5.19%	4.53%
HR-pQCT Radial Ct vBMD	1.16%	1.86%	1.55%
HR-pQCT Tibial Tb vBMD	0.89%	1.48%	1.24%
HR-pQCT Tibial Ct Thickness	2.65%	4.59%	3.88%
HR-pQCT Tibial Ct vBMD	1.11%	2.45%	2.06%
CTX	5.20%	11.00%	9.60%
P1NP	10.40%	22.00%	19.20%

Minimum difference detectable with 80% power, 5% two-tailed alpha calculated by T-test from constant (absolute) or our historical untreated.

Note that, while power analyses rely on simplified T-tests, the analysis plan will utilize ANCOVA, as described below. Power analyses based on the simplifying T-test assumptions are less efficient than the ANCOVA proposed here and covariate adjustment for baseline differences may allow detection of smaller differences than calculated by T-test. Thus, we view these calculated differences in absolute percentage points as conservative since the proposed statistical plan will incorporate covariate adjustment which should diminish error covariance, see below. Specific aims 2, 3 and 4 are here considered exploratory.

STATISTICAL METHODS
Given the non-randomized nature of this study, the primary objective will be to estimate the effect of denosumab treatment after cessation of TPTD from three perspectives:
1) **Within-group difference analysis**: A one-sample two-tailed T-test against the null hypothesis of no change will be used to assess whether denosumab treated patients differ on primary and secondary outcomes relative to the measurements acquired at the cessation of TPTD.
2) **Concurrent control analysis**: Analysis of covariance (ANCOVA) will be used to compare the prospectively followed denosumab patients to those who decline denosumab but continue observation after cessation of TPTD: covariates available at the end of TPTD treatment will be incorporated to reduce imbalances between the denosumab treated and untreated groups.
3) **Historical control analysis**: ANCOVA will be used to compare the prospectively treated denosumab patients to our historical cohort of 14 patients followed for 1-year after cessation of TPTD (see Sections 3C.6 and 3C.7); covariates available at the end of TPTD treatment will be incorporated to reduce imbalances between prospective and historical groups.

We will also compare the concurrent and historical control cohorts with respect to age, baseline BMD and response to TPTD. If the cohorts do not differ significantly from each other, we will combine the cohorts and analyze the combined cohorts as described below. As this is a small, non-randomized study, we will not be able to examine the comparative incidence of adverse events in treated and untreated subjects, nor will we be able to assess the comparative incidence of incident fractures. However, we will administer ADVERSE EVENTS and FRACTURE questionnaires at each study visit and will prospectively capture and code all Serious and Nonserious Adverse Events, with particular emphasis on those found to be associated with Denosumab in the pivotal Phase III clinical trials (see below). We will capture incident vertebral (by VFA scans at 12 and 24 months and radiographs of incident clinical vertebral fractures) and non-vertebral fractures (verified by review of radiographs or radiograph reports).

The main study analysis is defined by Analysis 1, while Analyses 2 and 3 are exploratory. For all analyses, the baseline measurements will be those acquired at the 24-month TPTD visit. The results of Analysis 1 will provide estimates of confidence limits surrounding this point estimate of the post-TPTD changes attributable to denosumab. Additionally, we will use repeated measures ANOVA to estimate the consistency of the trajectory of within-subject change in outcomes over the period of observation. This will be followed by a sensitivity analysis to identify the degree to which estimates of the average within-subject change attributable to denosumab is dependent on the influence of unusual or outlier responders. A responder analysis will be conducted to estimate the association between baseline and/or time-dependent variables on 2-year LS BMD change when this response is categorized into tertiles and examine with polymatous logistic regression. These confidence limits form, in the short term, the basis for estimating the likelihood of additional BMD improvement with denosumab in patients completing TPTD therapy, and, in the long-term, the basis for projecting the study design requirements of a future randomized study

Exploratory analyses for Analyses 2 and 3 will examine the repeated measures time-course of change with linear mixed models for repeated measures[29]. Fixed effects for group, time and their interaction, and empirically determined covariance structure selected prior to inferential testing, and time-dependent covariates for denosumab compliance and other medications will be entered as random effects. Baseline difference covariates, if any, will be entered as time-independent adjustments. The primary analysis of interest is the between-group difference in the group-by-time interaction. Secondary analyses of interest are the within-group differences between times and the between-group differences at comparably measured times. These analyses will employ the method of simultaneous confidence intervals calculated from the means and variances of the overall repeated measures within-subject model. Because for Analysis 2 and 3 we have repeatedly collected measures during the TPTD-treated phase, we can estimate the dependence of the direction and magnitude of the average within-subject change as the subject's bone metabolic activity shifts from that seen on TPTD (increased remodeling) to that typically seen on denosumab (decreased remodeling). If we were able to randomize subjects completing TPTD to denosumab, this comparison would follow traditional frequentist methods for randomized designs in which extended baseline information is available. In the absence of randomized allocation to denosumab, we propose to explore this valuable information with two non-parametric methods: regression discontinuity analysis (RDA) [30] and generalize additive models (GAM)[31]. RDA capitalizes on the fact that a cut-point value of the primary outcome at baseline can be selected where there will be some subjects who chose to take denosumab and some who chose only to be followed. To the extent that their course is dependent upon their baseline value and not other confounders, their different trajectories approximate an equi-likely allocation to different treatments. The RDA proceeds by analyzing the treated versus untreated group difference within a boundary width to the baseline cut-point. This "strawman" analysis is then subjected to challenge by examining the consistency of the initial estimate of the group difference as potential confounders are sequentially explored. We will pursue this approach by exploring a cut-off threshold of 6% for the TPTD-associated 12-month increase in LS-BMD, and, separately, a 2% increase in TH-BMD, to estimate treatment group differences in slopes and intercepts. Separately, GAM will be used to estimate the contribution of the TPTD longitudinal effects on bone and biochemistries to the shape of the denosumab-treated change in primary and secondary outcomes.

For Specific Aim 2, change in central QCT measures of bone structure from baseline to 12 months will be analyzed with ANCOVA of the change with covariates as described above. For Specific Aim 3, sequentially acquired HR-pQCT images and the ITS, cortical porosity and microFEA image analyses will be analyzed with linear mixed models for repeated measures, as described above. For Specific Aim 4, partial least squares regression (PLS)[32] with lagged-variables will be used to estimate the time dependency of changes in imaging-

based bone structure, quality and microarchitecture upon PTH and bone turnover marker concentrations and changes over the course of treatment.

3. Study Procedures

3.A. Calcium and Vitamin D, Fractures

All participants FD003902 are currently receiving calcium and multivitamin supplements. Supplements will continue to be provided during the new study: a daily multivitamin that contains 400 IU of vitamin D and very little or no calcium and a calcium supplement (Citracal +D), which contains 315 mg calcium and 250 IU vitamin per caplet. They will be instructed to take 3 Citracal +D daily (945 mg calcium and 750 IU vitamin D in total). Thus their total vitamin D intake from supplements will be 1150 IU daily. If their serum 25-OHD levels are < 30 ng/ml at Visit 1, additional vitamin D will be prescribed as clinically appropriate to maintain levels ≥ 30 ng/ml.

FRACTURES: We will capture all incident fractures by structured interview at each visit. Incident clinical and morphometric vertebral fractures will be ascertained by DXA Vertebral Fracture Analysis (VFA) scans at 12, 24 and 36 months. All subjects are instructed to notify their study team if they sustain a clinical vertebral fracture or a nonvertebral fracture. In addition, subjects will be specifically queried about incident fractures at each visit. All nonvertebral fractures will be verified by review of radiographs or radiograph reports.

STUDY PROTOCOL (Table 2)

Schedule of Visits

Visit 1: Recruitment, Protocol review, and Informed Consent: This will take place *during* FD003902 participation during a scheduled study visit in year 2. Renal and hepatic function and vitamin D status will be assessed, study procedures will be reviewed and informed consent obtained. If serum 25-OHD levels are < 30 ng/ml, supplementation will be prescribed and serum levels repeated to confirm efficacy (see CALCIUM AND VITAMIN D above).

Visit 2: Eligibility: Laboratory assessments to confirm study eligibility (aside from pregnancy testing) will take place one month before TPTD cessation/study enrollment, either locally or at the study site.

Visit 3 (Month 0): Enrollment Visit/TPTD Cessation/Denosumab Initiation: After 24 months of TPTD, subjects are seen for Year 2 assessments during FD003902 and costs for the imaging and biochemical studies are therefore not included in the budget for the extension study. At this visit, subjects undergo:

a) Oral inspection to assess for osteonecrosis of the jaw (ONJ)
b) Pregnancy testing
c) Imaging: DXA, VFA, HR-pQCT, cQCT
d) Laboratory assessments – plasma/serum collection/archive
e) **Denosumab administration #1**

Visit 4 (Month 3): Laboratory assessments – plasma/serum collection/archive

Visit 5 (Month 6):

a. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
b. Pregnancy testing
c. Laboratory assessments – safety labs and plasma/serum collection/archive
d. **Denosumab administration #2**

Visit 6 (Month 12):

a. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
b. Safety Labs (basic metabolic panel, calcium) may be obtained locally or at study site
c. Pregnancy testing
d. Imaging: DXA, VFA, HR-pQCT, cQCT
e. Laboratory assessments - plasma/serum collection/archive

f. **Denosumab administration #3**

Visit 7 (Month 18):

a. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
b. Pregnancy testing
c. Laboratory assessments – safety labs and plasma/serum collection/archive
d. **Denosumab administration #4**

Visit 8 (Month 24):

a. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
b. Pregnancy testing
c. Imaging: DXA, VFA, HR-pQCT
d. Laboratory assessments - plasma/serum collection
e. **Denosumab administration #5**

Visit 9 (Month 30):

e. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
f. Pregnancy testing
g. Laboratory assessments – safety labs and plasma/serum collection/archive
h. **Denosumab administration #6**

Visit 10 (Month 36):

f. Oral inspection to assess for osteonecrosis of the jaw (ONJ)
g. Pregnancy testing
h. Imaging: DXA, VFA, HR-pQCT
i. Laboratory assessments - plasma/serum collection

Table 2: Visit Protocol

	Protocol Review/ Informed Consent	Eligibility Labs (before visit)	Safety Labs (before visit*)	*Fractures and Adverse Events*	Pregnancy Testing *and Oral Exam*	Dmab	DXA spine VFA, HR-pQCT	cQCT	*Serum* for PTH, BTMs
Visit 1	X#								
Visit 2	X#	X#							
Visit 3			X#	X#	X#	X	X#	X#	X#
Visit 4			X	X					X
Visit 5			X	X	X	X@			X&
Visit 6			X	X#	X#	X	X#	X#	X#
Visit 7			X	X	X	X@			X&
Visit 8			X	X#	X#	X	X#		X#
Visit 9			X	X	X	X@			X&
Visit 10			X	X#	X#		X#		X#

* Safety labs may be obtained locally within 1 month of study visit
@ Denosumab may be administered at local physicians' offices if subject lives long distance from study site
& Plasma/serum collection may be done locally, shipped to CUMC if subject lives long distance from study site
# Only procedures required to be completed by the study control arm.

Recruitment: For FD003902, subjects are recruited by referral and advertisement. Our center has an extensive track record in recruitment of young women with IOP, and though recruitment has been slower than anticipated, we are confident that the enrollment goal of 41 women will be reached. Women will be recruited for

participation in this denosumab study DURING their participation in FD003902. We will inform subjects of the option to enroll in the denosumab study during FD003902 or at FD003902 enrollment. We will discuss study protocol, risks and benefits and perform consenting procedures for the extension study during the visit that takes place at the 18 month TPTD treatment time-point.

Denosumab Storage and Administration: Denosumab will be provided by Amgen (please see letter of endorsement). Denosumab medication will be shipped to the Research Pharmacy at CUMC where it will be stored according to industry standards and dispensed by the research pharmacies at Creighton University according to standard, IRB-approved procedures. Denosumab (Prolia®, 60mg, open label) will be administered to participants every 6 months, by subcutaneous injection performed according to standard procedures by licensed providers at Columbia University (Mary Ann Della Badia, RN) and Creighton University (Julie Stubby, RN). Denosumab may also be shipped from Columbia to study subjects' local physician's office and administered by licensed providers if the subject lives a long distance from either study site. To ensure that denosumab drug delivery is the same, standard operating procedures will be developed and reviewed with the administering physician, if it is necessary for local physicians to administer injections.

3.C. Study Procedures

3.1. BMD by DXA: BMD (aBMD) of the LS (L1-4), right proximal femur and non-dominant forearm will be measured on Hologic QDR Discovery densitometers (Hologic, Inc., Waltham, MA). At CUMC and Creighton, dedicated, licensed x-ray technicians with long-term research experience perform all scans. Phantoms are scanned daily to check for detector drift and the results are appended to a quality control (QC) database. Results are downloaded to specific project databases. QC procedures include circulation of phantoms biannually. At CUMC, precision is 0.86% for the LS, 1.36% for the FN, and 0.70% for the 1/3 radius. At Creighton, precision is 0.94% for the LS, 1.40% for the FN and 1.62% for the 1/3 radius. Radiation exposure for DXA of the spine, hip and forearm with the Hologic Discovery machine is 7.45 μSv.

3.2. Vertebral Fracture Assessment (VFA) by DXA: Visual semiquantitative identification of vertebral fractures is obtained from images acquired by fan-bean DXA scanners. VFA demonstrates good agreement with conventional radiographs (96.3%, k=0.79) in classifying vertebrae as normal or deformed. With its low radiation and good precision, VFA is useful to identify subjects with and without vertebral fractures. **:** Radiation exposure for VFA by the Hologic Discovery machine 5.2 μSv.

3.3. High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT): HR-pQCT is performed on the XtremeCT II (Scanco Medical AG, Switzerland). The nondominant distal radius and tibia are immobilized in a carbon fiber shell (158-160). The region of interest is defined on a scout film by manual placement of a reference line at the endplate of the radius or tibia; the first slice is 9.5 mm and 22.5 mm proximal to the reference line at the radius and tibia respectively. A stack of 110 parallel CT slices is acquired at the distal end of both sites using an effective energy of 40 keV, slice thickness of 82 μm, image matrix size 1024x1024, nominal voxel size of 82 μm. This machine provides a 3D image of 9 mm in the axial direction. HR-pQCT provides microstructural information (cortical and trabecular density, cortical thickness, trabecular number, thickness and separation) previously obtainable only by an invasive bone biopsy. We are one of only 7 US medical centers with this technology. For HRpQCT of the forearm and leg, the estimated local average skin dose is 5 μSv per scan, and the effective whole body dose is below 5 μSv per scan, since only a very small fraction of the distal forearm or leg is irradiated.

3.4. Volumetric BMD by Central QCT: Volumetric QCT acquisitions of the L1-L2 vertebrae (80 kVp, 140 mAs, 2.5 mm slice thickness, pitch=1.2, standard reconstruction algorithm, 3-Bar Image Analysis QCT Calibration Phantom (Image Analysis, Columbia, KY) will be carried out on a Siemens Biograph 40 Slice CT Scanner (Siemens Medical Solutions, Malvern, PA), located within CUMC's Kreitchman PET/CT Center. Scan data are archived to CD and forwarded to a central analysis site (UC San Francisco, Dept. of Radiology) for scan quality assurance and analysis. Spine CT images are analyzed with image analysis software developed by our collaborator and consultant, Thomas Lang, Ph.D. L1-L2 measures analyzed by this software include vBMD of

an integral compartment containing the vertebral body and posterior elements (viBMD), the areal BMD of this region obtained by computing its bone mineral content and dividing by the projected area in the AP plane (QCTaBMD), and the vBMD of a region containing almost all of the trabecular bone in the vertebral centrum (vtBMD). BMD data obtained on each scanner are converted to the calibration-phantom equivalent BMD. To ensure comparability of data obtained on different CT scanners used at CUMC, CT data are cross-calibrated using the Image Analysis Torso Quality Control phantom (Image Analysis, Columbia, KY) which is scanned multiple times on each system. Radiation exposure from a central QCT scan of the spine is 470 μSv, which is equivalent to 2 months of background radiation.

3.5. Biochemical and Hormonal Assays

Safety: Within 30 days before each denosumab injection, fasting morning blood samples for basic metabolic panel, including serum calcium, will be collected and processed at a commercial laboratory (Quest Diagnostics, Teterboro, NJ).

Mineral Metabolism and Bone Turnover: Fasting morning plasma/serum will be collected at CUMC, Creighton, or (at 3 month, 6 month, 18 month and 30 month visits) through arrangement with a local laboratory near the subject's residence. Specimens will be shipped frozen from Creighton University and other laboratories to CUMC, aliquoted and frozen at -80^{o} for batch analysis in the Biomarker Core of the Irving Institute for Clinical and Translational Research (CUMC CTSA), under the direction of Serge Cremers, PhD., PharmD. All assays are currently in place and inter- and intra-assay precision values are given in brackets after each test.

PTH is measured by a well-established total intact PTH IRMA (Scantibodies Laboratory, Inc., Santee, CA; 6.8%, 4.8%)**, serum calcium** by colorimetric assay (Cobas Integra 400 Plus, Roche Diagnostics, Indianapolis, IN; 3.5%, 0.99%), Procollagen type 1 N-terminal propeptide (**PINP)** by RIA (Immunodiagnostic Systems, Scottsdale, AZ; 8.3%, 6.5%), C-telopeptide (**CTX**) by CLIA (Immunodiagnostic Systems, Scottsdale AZ; 6.3%, 3.2%), %, **25-OHD** by RIA (Diasorin RIA, Stillwater, MN; 10.5%, 8.2%), and **Osteocalcin** by ELISA (Immunodiagnostic Systems, Scottsdale, AZ; 4%, 2%).

4. STUDY DRUGS OR DEVICES

Denosumab will be provided to subjects at no cost. Denosumab medication will be stored and dispensed by the research pharmacies at Columbia and Creighton Universities according to standard, IRB-approved procedures. CUMC will be the primary Research Pharmacy and will ship study drug to Creighton University's Research Pharmacy for subjects enrolled at Creighton. Denosumab (Prolia®, 60mg, open label) will be administered to participants every 6 months, by subcutaneous injection performed according to standard procedures by licensed providers at Columbia University (Mary Ann Della Badia, RN) and Creighton University (Julie Stubby, RN). Denosumab may also be shipped from Columbia to study subjects' local physician's office and administered by licensed providers if the subject lives a long distance from either study site.

5. STUDY SUBJECTS

The inclusion and exclusion criteria for FD003902 are presented first (indented), followed by those for the denosumab extension study.

Inclusion Criteria (at entry into FD003902)

- Premenopausal women, aged 20-45, with regular menses and no historical or biochemical secondary cause of osteoporosis; the lower age limit is to ensure epiphyses are fused, the upper to make it less likely that women will enter menopause during the study. All subjects under age 25 will be screened prior to enrollment to rule out open epiphyses.
- Documented adult fractures judged to be low-trauma (equivalent to a fall from a standing height or less) and/or T $\leq$ -2.5 or Z score $\leq$ -2.0 at the LS, FN or TH. Inclusion criteria vary slightly by age:
    - Premenopausal women ages 20-35 years must have at least one major osteoporotic fracture (excluding fractures of fingers, toes and face) AND low BMD defined as a T-score or Z-score ≤ -1.5.

- Premenopausal women above the age of 35 years must have a history of fracture AND/OR low BMD defined as Z-score < -2.0. Women above age 35 may also be enrolled on the basis of low BMD alone, without a history of low trauma fracture.
- Must agree to use effective contraception throughout the period of study drug administration

Exclusion Criteria (at entry into FD003902)

- History of any condition that increases the risk of osteosarcoma (Paget's disease, skeletal irradiation)
- Early follicular phase serum FSH>20 mIU/ml (to exclude perimenopausal women)
- Disorders of mineral metabolism: 1°/2° hyperparathyroidism, osteomalacia, osteogenesis imperfecta (OI) or Ehlers Danlos (ED). Subjects will not routinely undergo genetic testing for OI or ED as part of their screening evaluation. However, each subject will have a detailed medical and family history and a complete physical examination by one of the physician investigators. Women with historical features or physical examination findings suggestive of OI or ED will be referred for genetic evaluation. If the genetic evaluation is positive for OI or ED, the subject will be excluded from participation.
- Suspicion of osteomalacia (elevated alkaline phosphatase, bone pain exacerbated by weight bearing, bone tenderness)
- Vitamin D deficiency (serum 25-OHD<20 ng/ml). Women with levels of 10-20 ng/ml will be eligible after treatment with vitamin D has resulted in levels ≥20 ng/ml.
- Pregnancy or lactation within past 12 months
- Prolonged amenorrhea (≥ 6 months) during reproductive years (except pregnancy or lactation)
- Prior eating disorder (hypothalamic or exercise induced amenorrhea now resolved may be acceptable if symptoms occurred at age >20 years, for <1year, >5 years ago). The Eating Disorder Examination - Questionnaire is given to identify women with subclinical eating disorders[33,34]
- Malignancy, except cured basal or squamous cell skin carcinoma
- Endocrinopathy: untreated hyperthyroidism or hypothyroidism; Cushing's syndrome, prolactinoma
- Renal insufficiency (serum creatinine above upper limit of female normal range)
- Liver disease (AST, ALT, bilirubin, total alkaline phosphatase activity above upper normal limit)
- Intestinal disorders (celiac disease, pancreatic insufficiency, Crohn's Disease or ulcerative colitis
- History of or current GC therapy, anticonvulsants, anticoagulants, methotrexate, GnRH agonists to suppress menstruation
- Oral GC dose equivalent >5 mg prednisone for >3 mo. Inhaled GC exposure > 500 mcg daily for > 3 mo.
- Current anticoagulant use; past use of warfarin or low molecular weight heparin is not an exclusion
- Depo Provera (depot medroxyprogesterone acetate) unless taken after age 20, more than 5 years ago
- Drugs for osteoporosis (raloxifene, bisphosphonates, denosumab, calcitonin, TPTD). Subjects who discontinue these medications will be eligible 3 months after stopping raloxifene or calcitonin, 12 months after stopping alendronate, risedronate, ibandronate, or pamidronate and 18 months after stopping denosumab. Subjects with prior use of zoledronate may be eligible if received only one dose >4 years ago. Total bisphosphonate/denosumab exposure must be ≤ 1 year. Subjects who have taken TPTD in the past will not be eligible unless used for <3 months, > 2 years ago.

At FD003902 Study Entry, the Following Must Be Normal:

Blood count, Westergren ESR, electrolytes, creatinine, thyroid and liver function tests, uric acid, calcium, phosphate, 25-OHD (>20 ng/ml; those with levels between 21-29 ng/ml will be treated with supplementation to achieve levels >30), intact PTH (<65 pg/ml), tissue transglutaminase IgA antibody (tTG; 99% specific and 95% sensitive for celiac disease) and immunoglobulin A (IgA) to exclude celiac disease, 24-h urine for creatinine, calcium (<300mg/gCr), free cortisol. Other tests may be performed if less common etiologies are suspected.

DENOSUMAB STUDY INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria: All women completing at least 12 months of Forteo treatment under FD003902 who remain without a diagnosis of an excluded medical conditions and medication exposures as above, will be offered enrollment into this Phase IIB pilot study. Women who transitioned into menopause during FD003902 will be excluded from participation as this could affect the results of the study. Women who transition into menopause during the denosumab study will provide valuable information, but will be analyzed separately from those who remain premenopausal during the study.

Exclusion Criteria:

1) Known intolerance to calcium supplements
2) Contraindications or denosumab
    a. Hypocalcemia
    b. Pregnancy
    c. Known hypersensitivity to denosumab
3) History of osteomalacia
4) History of osteonecrosis of the jaw
5) History of dental extraction or other invasive dental surgery within the prior 6 months
6) Invasive dental work planned in the next 3 years
7) History of HIV, hepatitis C or hepatitis B infection
8) Any condition or illness (acute, chronic, or history), which in the opinion of the Investigator might interfere with the evaluation of efficacy and safety during the study or may otherwise compromise the safety of the subject
9) Self-reported or known alcohol or drug abuse within the previous 12 months
10) Current or recent (within 1 year of enrollment inflammatory bowel disease or malabsorption
11) Abnormal laboratory tests performed during Visit 1 (see below under STUDY PROTOCOL)
    a. Renal insufficiency or liver disease: Creatinine, AST/ALT above upper limit of normal
    b. Hypercalcemia, hypocalcemia
    c. Vitamin D deficiency: 25-OHD < 30 ng/mL

Subjects must be willing to participate voluntarily. Specifically excluded are the following: 1) women less than 20 (or 35 in the case of those who wish to participate because they have low BMD); 2) protected individuals (institutionalized); 3) prisoners; 4) any other prospective participant who, for any reason, might not be able to give voluntary informed consent.

Creighton University is a collaborating site. At Creighton University, Drs. Robert Recker and Joan Lappe will recruit subjects and perform the same studies as proposed for Columbia University. Dr. Thomas Lang will analyze central QCT scans at UCSF.

6. RECRUITMENT

Patients will be recruited from among participants in **FD003902** by the physician co-investigators and research coordinators. The Principal Investigators, Dr. Shane and Dr. Cohen, Co-investigators, Dr. Recker, and Dr. Lappe, and Research Coordinators, Mrs. Bucovsky, Ms. Kamanda-Kosseh and Mrs. Stubby, will be directly responsible for enrolling subjects and obtaining consent for the study. Permission will be first obtained from the patients' primary care physician, if appropriate. Written consent will always be obtained according to appropriate Informed Consent forms that will be reviewed and approved by the Institutional Review Boards of the both institutions. Each patient is counseled at the time of enrollment that all aspects of the study are separate from their management as a patient with osteoporosis. They are assured that participation is entirely voluntary and that refusal to participate in the study will not in any way influence their care. Statements to this effect will be included in all Informed Consent forms, which will be signed by the investigator obtaining consent and by the subject in the presence of a witness.

7. INFORMED CONSENT PROCESS

Written consent will always be obtained according to appropriate Informed Consent forms that will be reviewed and approved by the institutional review board of the Columbia University Medical Center. Potential

participants are assured that participation is voluntary and that refusal to participate will not influence their care. Statements to this effect will be included in all Informed Consent forms, which will be signed by the investigator or coordinator obtaining consent and by the subject. All investigators and coordinators have completed courses in Good Clinical Practices and HIPAA compliance. Written informed consent will be obtained for every subject by the investigators after an explanation of the purpose, risks and benefits of the study. Confidentiality will be guarded with the use of computers that are password protected and storing questionnaires with sensitive information within a locked file. All subjects will be provided with instructions on how to contact the investigative team if any problems or concerns arise.

The informed consent process will be conducted by a study investigator or research coordinator prior to initiation of study-related procedures. Subjects will have an opportunity to asked questions prior to signing the form, and all participants will receive a copy of the signed consent form for their records.

8. CONFIDENTIALITY

Confidentiality of patient data in this project will be ensured. Personal Identifying Health Information (PHI) of participants will be kept only in secure files accessible to the PI, investigators and project coordinators. Data will be recorded on case report forms on which the only identifier is a research ID code. Only the PI and project coordinator have access to the link between the research ID code and PHI. No names or identifying information will be included in research reports. Subjects' names will not appear on questionnaires. All computers housing research data have passwords and timed screen savers requiring a password for access. Through these safeguards, the confidentiality of the data will be ensured.

Information obtained in the setting in this study may be made available to the following entities:
- The investigator, study staff and other health professionals who may be evaluating the study
- Columbia University
- New York Presbyterian Hospital
- Authorized representatives of the Food and Drug Administration ('FDA'), the Office of Human Research Protections ('OHRP') or other government regulatory agencies
- Applicable Institutional Review Boards ('IRBs') that independently review the study to assure adequate protection of research participants, as required by federal regulations.

The investigator, regulatory authorities, IRB and study sponsor may keep the research records indefinitely. If the results of the study are published or presented at a medical or scientific meeting, subjects will not be identified.

9. PRIVACY PROTECTIONS

We will take all necessary steps to safeguard each participant’s expectation that the information they offer will be held in confidence. These protections will apply to all research related data collection and procedures, as well as to all forums

Only a select group of study personnel will have access to patient study files. We are fully committed to safeguarding an individual’s expectation that the information they offer will be held in confidence. All subjects will sign a HIPAA form in addition to the informed consent document to prevent inappropriate use or any disclosure of individuals' health information and to require any organizations which use health information to protect that information and the systems which store, transmit, and process it. The subject has the right to revoke the authorization for us to access her health information at any time, as is stated in the HIPAA form that each subject will sign prior to participation.

Our safety reporting responsibilities require the investigator to report any adverse events to the IRB, and the FDA. These reports will include only a study ID, and will not divulge the participant’s identity.

10. POTENTIAL RISKS
The risks of this study are related to the venipuncture, radiation exposure and the study medication, denosumab.

Venipuncture: The risks of venipuncture for blood drawing include pain, bleeding, bruising, and a remote possibility of infection or inflammation at the site. Additionally, there is a possible risk of syncope in individuals who are prone to vasovagal responses. To minimize these risks, trained phlebotomists who follow proper technique perform all venipunctures.

Radiation: Radiation exposure for DXA of the spine, hip and forearm with the Hologic Discovery machine is 7.45 µSv and for VFA 5.2 µSv. This is about the amount the average person receives from background radiation in 7 days. For HRpQCT of the forearm and leg, the estimated local average skin dose is 5 µSv per scan, and the effective whole body dose is below 5 µSv per scan, since only a very small fraction of the distal forearm or leg is irradiated. Radiation exposure from a central QCT scan of the spine is 470 µSv, which is equivalent to 2 months of background radiation. Based on these data, we estimate the following radiation exposure for participation in this clinical trial, primarily driven by the additional central QCT measurements of spine volumetric BMD at 12 month of denosumab, is 538 µSv, approximately equivalent to 2.2 months of background radiation. For purposes of comparison, there is 60 µSv radiation associated with a round-trip transcontinental plane flight and 2400-3600 µSv of natural background radiation in a year. The amount of radiation from exposure associated with a standard abdominal/pelvic or chest CT scan is 7,000-10,000 µSv. Expressed as equivalencies to background radiation, a standard mammogram is associated with radiation exposure equivalent to approximately 2 to 3 months of natural background radiation. A standard abdominal or chest CT scan is associated with radiation exposure equivalent to approximately 15-22 months of natural background radiation. Thus, the amount of radiation that would be received by participation in the entire study (including both the TPTD and the denosumab phases) is less than that from a standard CT scan of the chest or abdomen. We will counsel all study subjects about the total amount of radiation that they will receive as a result of participation as part of Informed Consent procedures of that particular study. In addition, they will be counseled that radiation exposure is cumulative throughout life and any additional exposure should be considered carefully.

Denosumab: Denosumab, a RANK L inhibitor, is approved for the treatment of men and postmenopausal women with osteoporosis who are at high risk for fracture. Clinical trial experience and clinical use since its approval in June, 2010, show that the drug appears to be well tolerated. The dose we will use in this study is the standard approved dose for postmenopausal and male osteoporosis, namely 60 mg every 6 months.

As of 26 September 2015, approximately 23,148 subjects have received denosumab 60mg in research studies. Since it was approved for sale in 2010, the worldwide use as of 26 September 2015 includes approximately 4.5 million people who have been prescribed denosumab (Prolia®) for treatment. Side effects that other people with osteoporosis or bone loss due to some cancer treatment had in research studies that are thought to have been caused by denosumab 60 mg are:

Very Common side effects (which may affect more than 1 person in 10):
-joint pain
-pain in extremity

Common side effects (which may affect between 1 and 10 people in every 100):
-cataracts
-cough
-decreased skin sensation
-dizziness
-difficulty emptying the bladder
-eczema
-low blood calcium (Hypocalcemia)
-muscle and bone pain

-osteoarthritis
-reports of high cholesterol

Uncommon side effects (which may affect between 1 and 10 people in every 1,000):
-skin infections leading to hospitalization
-broken bones in your spine after stopping denosumab

Rare side effects (which may affect between 1 and 10 people in every 10,000):
-allergic reaction (drug hypersensitivity)
-osteonecrosis of the jaw (ONJ)
-unusual thigh bone fracture (atypical femoral fractures)

Hypocalcemia: Denosumab can lower serum calcium levels, and cause symptomatic hypocalcemia, particularly in patients who are vitamin D deficient or have stage 4 chronic kidney disease (CKD). However, patients with CKD are excluded from participation, serum 25-OH vitamin D must be $\geq$ 30 ng/ml prior to entry, and all participants will be instructed to take 945 mg calcium daily or supplements, and to take vitamin D 1150 IU daily (in calcium and multivitamin supplements). Therefore, the risk of symptomatic hypocalcemia is likely to be very low in participants in this study. We will measure serum calcium within a 30-day period before each denosumab dose, and denosumab will not be administered if the serum calcium is below the lower limit of the laboratory normal range. In addition, all patients will be educated about symptoms of hypocalcemia (spasms, tremors, twitches, muscle cramp, and numbness or tingling around the mouth, fingers or toes). Participants will be instructed to seek medical attention if they experience any of these symptoms.
Hypersensitivity: Clinically significant hypersensitivity including anaphylaxis has been reported with denosumab. Symptoms have included hypotension, dyspnea, throat tightness, facial and upper airway edema, pruritis, and urticaria. The majority of injections will be given at CUMC or Creighton and subjects who receive their injections locally will be have them performed at a physician's office by a physician or nurse. Participants will be instructed to call their physician or go immediately to the closest Emergency Room if an anaphylactic or other clinically significant allergic reaction occurs. Participants who develop clinically significant hypersensitivity will be withdrawn from the study.
Serious Infections: In a clinical trial (N = 7808) in women with postmenopausal osteoporosis, serious infections leading to hospitalization were reported more frequently in the denosumab group than in the placebo group. Serious skin infections, as well as infections of the abdomen, urinary tract and ear, were more frequent in patients treated with denosumab. Endocarditis was also reported more frequently in denosumab-treated patients. The incidence of opportunistic infections and the overall incidence of infections were similar between the treatment groups. Although the post-marketing surveillance data have been reassuring in regard to the incidence of serious infection, all participants will be counseled about this possibility during informed consent procedures and instructed to seek prompt medical attention if they develop signs or symptoms of severe infection, including cellulitis. If participants develop serious infections during the trial, we will assess the advisability of continued denosumab therapy and will consider withdrawal from the study.
Dermatologic Adverse Reactions: In the same clinical trial in women with postmenopausal osteoporosis, epidermal and dermal adverse events such as dermatitis, eczema and rashes occurred at a significantly higher rate with denosumab compared to placebo. Most of these events were not specific to the injection site. All participants will be counseled about this possibility during informed consent procedures and instructed to seek prompt medical attention if they develop signs or symptoms of dermatitis, eczema and rashes. If participants develop dermatitis, eczema and rashes during the trial, we will assess the advisability of continued denosumab therapy and will consider withdrawal from the study.
Osteonecrosis of the Jaw (ONJ): ONJ, which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing, and has been reported in patients receiving denosumab. However, it is generally limited to patients with malignancy who are receiving the higher doses prescribed for prevention of skeletal related events and it is very rare in the doses used to treat osteoporosis, particularly in patients treated for <3 years, as will be the case in this study. An oral exam will be performed by the nurse- or physician-investigator (Dr. Shane or Dr. Cohen at Columbia, Dr. Recker or Dr. Lappe at Creighton) prior to initiation of denosumab and at each study visit to assess for ONJ. All participants will be counseled about this possibility during informed consent procedures and instructed to call the study team if they develop symptoms

of ONJ. All will be instructed to maintain good oral hygiene during treatment with denosumab. Women with a history of dental extraction or other invasive dental surgery within the prior 6 months or who require invasive dental work in the next 2 years will not be enrolled. For study participants who require invasive dental procedures during the study, the dentist or oral surgeon will be informed that they are receiving denosumab.

There may be a higher risk of ONJ in patients who took denosumab for a longer period of time.

Atypical Femoral Fractures: Atypical low-energy, or low trauma fractures of the femur shaft have been reported in a very small number of patients receiving denosumab for osteoporosis (approximately 2). Although this is very rare, particularly with short-term use (<5 years), as will be the case in this study, and causality has not been established, all participants will be counseled about this possibility during informed consent procedures and instructed to call the study team if they develop new or unusual thigh, hip, or groin pain. Any participant who develops thigh or groin pain will be evaluated to rule out an incomplete femur fracture. If a complete or incomplete atypical femoral fracture develops in a study participant, denosumab will be discontinued and the participant will be withdrawn from the study.

Other Adverse Reactions: Other adverse reactions reported in patients receiving denosumab include back pain, extremity pain, musculoskeletal pain, arthralgia, cystitis, nasopharyngitis and pancreatitis. In women with postmenopausal osteoporosis, the overall incidence of new malignancies was 4.3% in the placebo group and 4.8% in the denosumab groups. In men with osteoporosis, new malignancies were reported in no patients in the placebo group and 4 (3.3%) patients in the denosumab group. A causal relationship to drug exposure has not been established. All participants will be counseled about these possible adverse reactions during informed consent procedures and instructed to call the study team if they develop any unusual symptom or condition. All subjects will complete an adverse reaction questionnaire at each study visit. Adverse reactions will be tabulated and reported to both institutional IRBs, the DSMB and the FDA, as appropriate.

Injections site reaction. There may be a reaction near the area where the denosumab 60 mg is injected. In general, symptoms may include redness, tenderness or pain, bruising, warmth, swelling, itching, and/or infection at the injection site**.**

Children and adolescents. Denosumab has not been studied in newborns, children and adolescents; therefore, the effect on bones and teeth before normal bone growth is completed is not known. In studies of monkeys whose bones were still growing denosumab caused abnormalities in the bone where growth occurs (growth plate). As a result, this part of the bone may be more prone to injury and bone length may be reduced in people whose bones are still growing. An agent similar to denosumab administered to rats soon after birth also affected the growth plate resulting in bones not growing properly. This agent also prevented teeth from surfacing from the gums and caused the roots of the teeth to grow abnormally.

High blood calcium in children and adolescents after stopping denosumab. Some children and adolescents who were still growing during treatment with denosumab had an increase in blood calcium after stopping denosumab. Symptoms of high blood calcium may include stomach upset, nausea, vomiting, headache, and decreased alertness. High blood calcium may also lead to dehydration and kidney failure if not treated as soon as possible.

Teratogenicity: In contrast to bisphosphonates, denosumab does not accumulate in the skeleton. Therefore, teratogenic effects, if any, would essentially be limited to the period of administration. Thus, it is a more attractive choice for treating osteoporosis in women of childbearing age, as it is believed that there would be no potential for subsequent pregnancies to be affected by residual drug. However, there may be pregnancy-related risks in this population. Denosumab carries a category X in pregnancy rating from the FDA due to toxic effects in pregnant primates who received high doses at multiple times points throughout pregnancy. No data are available regarding effects on a human pregnancy. Therefore, we will take every precaution to ensure the safety of the use of this medication in these premenopausal women. Currently, subjects undergo urine pregnancy testing at EVERY imaging and drug dispensation visit for our studies of TPTD. The Informed Consent document they will sign requires them to use 2 highly effective forms of birth control (such as injectable contraceptives, IUDs, oral contraceptives or a vasectomized partner AND a condom) throughout the study and until 6 months after the last denosumab dose, as specified by our IRBs. We will counsel all participants about all available pregnancy risk data, including studies in primates, as described above. There is still a risk that pregnancy could occur despite the responsible use of a reliable method of birth control. All participants will agree to notify the investigator as soon as possible of any failure of proper use of birth control, or if they become pregnant, either of which will result in them being withdrawn from the study. If they become

pregnant, they will be asked to follow-up with the study team until the end of the pregnancy and up to thirty days after delivery. If a pregnancy occurs in a female subject, while the subject is taking denosumab, we will report the pregnancy to Amgen to Amgen's Pregnancy Surveillance Program within 10 business days of our awareness. In addition to reporting any pregnancies occurring during the study, we will monitor for pregnancies that occur after the last dose of denosumab through 6 months after the last dose. These precautions will prevent any cases of lactation on denosumab, as any women who become pregnant despite these precautions will be withdrawn from the study.

PREGNANCY AND LACTATION REPORTING PROCEDURES: *We will report pregnancy cases to Amgen's Pregnancy Surveillance Program within 10 business days of our awareness.*
Amgen's Pregnancy Surveillance Program contact information follows:
Phone: 1 800-77-Amgen (1-800-772-6436)
Website: www.amgenpregnancy.com

Drug-Induced Liver Injury (DILI)

The FDA Drug-induced Liver Injury (DILI) Guidance (http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf), the data suggest that the rate of DILI is relatively low (1/10,000) or less and that marked ALT elevations, particularly in association with increases in the total serum bilirubin not explained by any other cause, should prompt concern, as should signs and symptoms of hepatic dysfunction, including anorexia, nausea, vomiting, fatigue, right upper abdominal discomfort, jaundice). In response, we have added safety labs to each visit, including an hepatic function panel that includes albumin, transaminases, total and direct bilirubin and total alkaline phosphatase to the protocol at the baseline (pre-denosumab) visit and each subsequent visit (3M, 6M, 12M, 18M, 24M) that will be processed by Quest Diagnostics in real time and reviewed by research staff, including the study physician. We will develop and include in the Case Report Form, pages designed to capture information pertinent to the evaluation of treatment-emergent liver abnormalities, precisely as outlined in the FDA Guidance on Pages 13-14. All subjects must have normal liver function tests to be enrolled in the study. Any abnormalities in the ALT will be handled as follows (see Pages according to the following hepatotoxicity stopping and rechallenge rules: Any subject with an increase in serum ALT to >3UNL and/or serum total bilirubin >2UNL will:

- Have repeat testing within 48 to 72 hours of the receipt of test results by the site study coordinator to confirm the abnormalities and determine whether they are increasing. This testing may be done locally as many subjects live a long distance from the study site and normal ranges will be recorded.
- Be questioned specifically about symptoms of hepatic dysfunction, including anorexia, nausea, vomiting, fatigue, right upper abdominal discomfort, jaundice.
- If above abnormalities are confirmed, close observation will be initiated that will include:
    - Repeating liver enzyme and serum bilirubin tests two or three times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
    - Obtaining a more detailed history of symptoms and prior or concurrent diseases.
    - Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
    - Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
    - Obtaining a history of exposure to environmental chemical agents.
    - Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
    - Gastroenterology or hepatology consultation will be initiated immediately upon confirmation of results.
- Although transient fluctuations of ALT or AST are common, and progression to severe DILI or acute liver failure is uncommon, we feel that continuing denosumab would be unacceptably dangerous if there is persistent (> 2 weeks) or increasing serum ALT elevation or evidence of functional impairment, as indicated by rising bilirubin or INR, which represent substantial liver injury. Therefore, the drug will be discontinued if such laboratory abnormalities are documented during close observation.

11. DATA AND SAFETY MONITORING

A Data & Safety Monitoring Board has been established for the parent TPTD study (FD003902) and will be invited to continue for the denosumab study to ensure continuity. The DSMB will monitor subject accrual, ethical conduct of research, as well as oversee adverse events and any unforeseen consequences in the study population. The DSMB is chaired by Dr. Mishaela Rubin, an endocrinologist and Assistant Professor of Medicine at CUMC with expertise in osteoporosis and clinical trials, Dr. Judith Korner, an endocrinologist and Associate Professor of Medicine at CUMC outside of the field of metabolic bone diseases (obesity and weight control), and a statistician, Dr. Emelia Bagiella, Professor of Health Evidence and Policy at Mount Sinai Medical School in New York City. The DSMB is independent of all study personnel, and has signed a conflict of interest form to that effect. It meets annually face-to-face and by conference call as necessary in case of complications or issues. The DSMB may recommend any steps necessary to protect the participants. We will invite the current DSMB to monitor this new study as well, as there is such overlap between the two studies.

Interim Analysis: No efficacy interim analysis is planned. The proposed recruitment of a modest number of subjects in each group, with the anticipated recruitment schedule, diminishes the need for an interim efficacy analysis. However, this decision will be reviewed at the first meeting of the DSMB. Performance data will be evaluated. The first interim look at performance will occur when the initial 20 participants have completed the 6-month follow-up visit; thereafter interim reviews will occur at 6-month intervals.

Stopping Rules: Stopping rules will be invoked if new information emerges making the questions in this proposal moot or new information provides evidence suggesting that the use of denosumab in premenopausal women with IOP is contraindicated.

Protocol Modifications: Modifications will not be undertaken without notification of the IRB, the DSMB and the FDA Program Officer for the grant.

Reporting: All adverse events will be reported according to the guidelines set forth by the FDA and applicable IRBs. We commit to follow the FDA reporting requirements set forth in the Code of Federal Regulations Title 21, Section 312.32, which is summarized below.

The sponsor shall notify FDA and all participating investigators in a written IND safety report of:
(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or
(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than *15 calendar days* after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format and shall bear prominent identification of its contents.

The sponsor shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than *7 calendar days* after the sponsor's initial receipt of the information. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

The Principal Investigator will ensure that the FDA is informed of actions, if any, taken by the IRB as a result of its continuing review and summary reports will be submitted annually to the FDA. All adverse events that meet the criteria of Columbia University (CU) IRB's reporting policy are reportable to the IRB (link to policy is included below). The CU IRB requires reporting of all unanticipated problems considered to be unexpected, related or possibly related to participation in the research, and that suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized. The timeliness of this report will depend on whether the event is considered to be serious and whether it occurred at an internal or external site. Annual reports will be submitted to the IRB documenting all adverse events and unanticipated problems. Individual serious adverse events will be reported to the IRB and DSMB within 1 week. The PI will forward the report to the IRB and FDA devoid of patient-specific information.

Columbia University IRB Reporting Policy:
http://www.cumc.columbia.edu/dept/irb/policies/documents/UnanticipatedProblemsPolicy.FINALVERSION.012408.pdf)

Creighton University IRB Reporting Policy
http://www.creighton.edu/fileadmin/user/ResearchCompliance/IRB/Policies_and_Procedures/120_Unanticipated_Problems_Involving_Risks_to_Participants_or_Others.pdf

12. POTENTIAL BENEFITS
The IOP subjects may or may not benefit directly from this study. If patients respond to the medication they receive, they will benefit by improving their bone architecture, bone strength, and resistance to fractures.

On a wider scale, the information gained from this research project may apply to all women who have idiopathic osteoporosis and may directly impact upon the future treatment of this puzzling disorder.

13. ALTERNATIVES
The alternative is not to participate in this trial. If a subject decides to withdraw, she will be counseled about other, alternative osteoporosis treatment options. Alternate therapies include, but are not limited to, Alendronate (Fosamax), Risendronate (Actonel), Ibandronate (Boniva), Zometa (Reclast).

14. RESEARCH AT EXTERNAL SITES
Research for this protocol will be conducted at Columbia University Medical Center in NY, NY and at Creighton University Medical Center in Omaha, NE. The research team at Creighton University will be responsible for securing IRB approval for all research activities conducted at their site related to this protocol. We will maintain current versions of all IRB approvals and approved documents (ie – consent forms, etc) at our site and submit any changes promptly to the CUMC IRB. Our plan for data and safety monitoring will include monitoring activities and outcomes at both study sites.

15. COLUMBIA AS LEAD INSTITUTION
Columbia University will be considered the lead institution in this protocol. There will be one other site, Creighton University. As stated in the prior section, we will commit to the following: 1) obtain and maintain IRB approval at the Creighton site; 2) ensure that the Creighton site follows consent procedures and utilizes consent documents approved by their IRB (if the designated IRB is not the CU IRB, then the IRB-approved consent document must be similar to the CU IRB-approved consent document with regards the content and style of the document).

Creighton University's Federal-Wide Assurance # is FWA00001078.

16. Sponsor Responsibilities
Study compliance and subject safety will be monitored periodically by the sponsor-investigator, Dr. Elizabeth Shane. The task of overseeing subject safety will be the joint responsibility of both the DSMB as well as the task of the study investigators. Adverse events and/or unanticipated problems will be reported to the Columbia IRB in accordance with their policy and to the FDA in accordance with their reporting guidelines (see Section 11.0 for more detailed information). Study compliance and assurance that the study is conducted in accordance with the protocol will be facilitated by a protocol and operations manual developed by the investigator. Participating research personnel will be instructed in proper forms completion and electronic data entry procedures, as it is applicable to their study role. Adherence with the study protocol and recruitment goals will be monitored by the sponsor on an ongoing basis, with monthly conference calls made to participating sites (Creighton University). Upon identification of incomplete reports or missing data, this information will be obtained from the study site in writing.

It is the responsibility of the sponsor-investigator to submit all amendments, IND safety reports and annual reports to the FDA in compliance with the relevant institution's policies for reporting. Amendments to the

protocol must be approved by the IRB prior to the implementation of any changes to the protocol or consent forms, including, but not limited to, study questionnaires, procedures, and recruitment methods.

Records and documentation pertaining to the protocol will be kept in the protocol regulatory binder and online through the Columbia RASCAL system. Subject specific records will be stored in a password protected database or in a locked cabinet to ensure privacy and confidentiality, as is detailed in Sections 8.0 and 9.0. Data entry will be ongoing throughout the course of the protocol so that interval reports to the investigator, IRB and other regulatory bodies are readily accessible.

17. Literature Cited.

1. Cohen A, Recker RR, Lappe J, et al. Premenopausal women with idiopathic low-trauma fractures and/or low bone mineral density. Osteoporos Int 2012;23:171-82.
2. Cohen A, Dempster DW, Recker RR, et al. Abnormal bone microarchitecture and evidence of osteoblast dysfunction in premenopausal women with idiopathic osteoporosis. J Clin Endocrinol Metab 2011;96:3095-105.
3. Cohen A, Lang TF, McMahon DJ, et al. Central QCT reveals lower volumetric BMD and stiffness in premenopausal women with idiopathic osteoporosis, regardless of fracture history. J Clin Endocrinol Metab 2012;97:4244-52.
4. Cohen A, Liu XS, Stein EM, et al. Bone microarchitecture and stiffness in premenopausal women with idiopathic osteoporosis. J Clin Endocrinol Metab 2009;94:4351-60.
5. Cohen A, Stein EM, Recker RR, et al. Teriparatide for idiopathic osteoporosis in premenopausal women: a pilot study. J Clin Endocrinol Metab 2013;98:1971-81.
6. Nishiyama K, Wang J, Young P, et al. Teriparatide Is Associated with Improved Microarchitecture and Estimated Bone Strength in Premenopausal Women with Idiopathic Osteoporosis: An HR-pQCT Study. American Society for Bone and Mineral Research Annual Meeting; 2013; Baltimore, MD: American Society for Bone and Mineral Research.
7. Cosman F, Nieves J, Woelfert L, et al. Parathyroid hormone added to established hormone therapy: effects on vertebral fracture and maintenance of bone mass after parathyroid hormone withdrawal. J Bone Miner Res 2001;16:925-31.
8. Lane NE, Sanchez S, Modin GW, Genant HK, Pierini E, Arnaud CD. Parathyroid hormone treatment can reverse corticosteroid-induced osteoporosis. Results of a randomized controlled clinical trial. J Clin Invest 1998;102:1627-33.
9. Lane NE, Sanchez S, Modin GW, Genant HK, Pierini E, Arnaud CD. Bone mass continues to increase at the hip after parathyroid hormone treatment is discontinued in glucocorticoid-induced osteoporosis: results of a randomized controlled clinical trial. J Bone Miner Res 2000;15:944-51.
10. Finkelstein JS, Arnold AL. Increases in bone mineral density after discontinuation of daily human parathyroid hormone and gonadotropin-releasing hormone analog administration in women with endometriosis. J Clin Endocrinol Metab 1999;84:1214-9.
11. Orwoll E, Teglbjaerg CS, Langdahl BL, et al. A randomized, placebo-controlled study of the effects of denosumab for the treatment of men with low bone mineral density. J Clin Endocrinol Metab 2012;97:3161-9.
12. Black DM, Bilezikian JP, Ensrud KE, et al. One year of alendronate after one year of parathyroid hormone (1-84) for osteoporosis. N Engl J Med 2005;353:555-65.
13. Kaufman JM, Orwoll E, Goemaere S, et al. Teriparatide effects on vertebral fractures and bone mineral density in men with osteoporosis: treatment and discontinuation of therapy. Osteoporos Int 2005;16:510-6.
14. Kurland ES, Heller SL, Diamond B, McMahon DJ, Cosman F, Bilezikian JP. The importance of bisphosphonate therapy in maintaining bone mass in men after therapy with teriparatide [human parathyroid hormone(1-34)]. Osteoporos Int 2004;15:992-7.
15. Leder B, Tsai J, Uilein A, et al. The transition from denosumab to teriparatide or from teriparatide to denosumab in postmenopaual women with osteoporosis: The DATA-SWITCH study. American Society for Bone and Mineral Research 2014 Annual Meeting. Houston, TX: Wiley; 2014.
16. Bone HG, Bolognese MA, Yuen CK, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. J Clin Endocrinol Metab 2008;93:2149-57.

17. Genant HK, Libanati C, Engelke K, et al. Improvements in hip trabecular, subcortical, and cortical density and mass in postmenopausal women with osteoporosis treated with denosumab. Bone 2013;56:482-8.
18. Keaveny T, McClung M, Genant H, et al. Femoral and vertebral strength improvements in postmenopausal women with osteoporosis treated with denosumab. J Bone Miner Res 2013.
19. McClung MR, Zanchetta JR, Hoiseth A, et al. Denosumab densitometric changes assessed by quantitative computed tomography at the spine and hip in postmenopausal women with osteoporosis. J Clin Densitom 2013;16:250-6.
20. Keaveny TM, Donley DW, Hoffmann PF, Mitlak BH, Glass EV, San Martin JA. Effects of teriparatide and alendronate on vertebral strength as assessed by finite element modeling of QCT scans in women with osteoporosis. J Bone Miner Res 2007;22:149-57.
21. Keaveny TM, McClung MR, Wan X, Kopperdahl DL, Mitlak BH, Krohn K. Femoral strength in osteoporotic women treated with teriparatide or alendronate. Bone 2012;50:165-70.
22. Seeman E, Delmas PD, Hanley DA, et al. Microarchitectural deterioration of cortical and trabecular bone: differing effects of denosumab and alendronate. J Bone Miner Res 2010;25:1886-94.
23. Kostenuik PJ, Smith SY, Jolette J, Schroeder J, Pyrah I, Ominsky MS. Decreased bone remodeling and porosity are associated with improved bone strength in ovariectomized cynomolgus monkeys treated with denosumab, a fully human RANKL antibody. Bone 2011;49:151-61.
24. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 2006;354:821-31.
25. Papapoulos S, Chapurlat R, Libanati C, et al. Five years of denosumab exposure in women with postmenopausal osteoporosis: results from the first two years of the FREEDOM extension. J Bone Miner Res 2012;27:694-701.
26. Bone HG, Bolognese MA, Yuen CK, et al. Effects of denosumab treatment and discontinuation on bone mineral density and bone turnover markers in postmenopausal women with low bone mass. J Clin Endocrinol Metab 2011;96:972-80.
27. Tsai JN, Uihlein AV, Lee H, et al. Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: the DATA study randomised trial. Lancet 2013;382:50-6.
28. Seeman E, Libanati C, Austin M, Chapurlat R, Boyd SK, Zebaze RM. The Transitory Increase in PTH Following Denosumab Administration is Associated With Reduced Intracortical Porosity: a Distinctive Attribute of Denosumab Therapy. American Society for Bone and Mineral Research Annual Meeting; 2011; San Diego, CA.
29. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13-22.
30. Thistlewaite D, Campbell d. Regression-Discontinuity Analysis: An alternative to the ex post facto experiment. Journal of Educational Psychology 1960;51:309-17.
31. Hastie TJ, Tibshirani RJ. Generalized additive models (with discussion). Statistical Science 1986;1:297-318.
32. Wold S. PLS for Multivariate Linear Modeling. QSAR: Chemometric Methods in Molecular Design Methods and Principles in Medicinal Chemistry 1994.
33. Cooper Z, Cooper P, Fairburn C. The validity of the eating disorder examination and its subscales. British Journal of Psychiatry 1989;154:807-12.
34. Black C, Wilson G. Assessment of eating disorders:interview versus questionnaire. Int J Eat Disord 1996;20:43-50.