Mechanisms Underlying the Bone Modeling Effects of Combined Anabolic/Antiresorptive Administration

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I. Statement of Compliance

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the NIAMS Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

II. Background and Significance

A. Historical background

In the United States, osteoporosis affects over 20 million women and men and 1.5 million Americans experience a fragility fracture every year.[1] One in five women will experience a hip fracture in their lifetime, a devastating consequence that conveys a 24% excess 1-year mortality.[2,3] Current osteoporosis therapies increase bone mineral density (BMD) modestly and reduce fracture incidence in high-risk populations, but their efficacy is limited, especially in the appendicular skeleton where fracture reduction does not exceed 50% for any agent.[4-7] The real world utility of these drugs is further limited by poor adherence and safety concerns.[8-10]

Osteoporosis medications can be separated into two categories: (1) antiresorptive medications such as the nitrogen-containing bisphosphonates and the receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor, denosumab, and (2) anabolic agents such as teriparatide (parathyroid hormone (PTH)-1-34) and abaloparatide. Over the past decade, several groups have investigated the efficacy of combining anabolic agents with bisphosphonates but no combination was shown to increase bone mass more than the individual monotherapies.[11-14] In contrast to results with these anabolic/bisphosphonate combinations, we recently reported in the Denosumab And Teriparatide Administration (DATA) study that concurrent denosumab and teriparatide increased spine and hip BMD and improved bone microarchitecture more rapidly and to a greater extent than either drug alone.[15,16] The mechanistic basis for the efficacy of this combination, however, has not yet been defined. Based on the changes in biochemical markers of bone turnover observed in DATA, we have hypothesized that the combination may derive its efficacy from denosumab's ability to fully block the pro-resorptive effects of teriparatide while still allowing for teriparatide to stimulate modeling-based bone formation. We now aim to define the mechanisms that form the basis of these novel clinical observations. In so doing, we will build a framework for the design of robust clinical trials, including fracture prevention outcomes, with the potential to fundamentally advance osteoporosis treatment.

Effects of teriparatide on bone metabolism and histomorphometry: Teriparatide, through its binding to the PTH/PTHrP receptor stimulates bone remodeling by increasing the activity of both osteoclastic bone resorption and osteoblastic bone formation (with intermittent administration favoring bone formation).[17,18] The effects on remodeling peak at 3-12 months, after which rates revert towards baseline.[7,11,13,15] Additionally, PTH and its analogs influence the activity of other bone cells including osteocytes (decreasing the expression of the *sost* gene that encodes the osteoblast inhibitor, sclerostin and increasing the expression of RANKL) and lining cells (activating) though the relative physiologic importance of each of these activities is not clear.[19,20] In humans, teriparatide has been shown to increase BMD at the hip and spine and reduce the risk of both spine and non-

spine fractures in postmenopausal women.[7,21-26] In iliac crest bone biopsy specimens from teriparatide-treated women, there are detectable increases in bone formation rates (BFR) and mineralizing surface/bone surface (MS/BS) as early as 1 month after the treatment initiation in all four bone compartments (cancellous, endocortical, intracortical, and periosteal).[21,27,28] Pro-resorptive effects, including increasing cortical porosity, have also been consistently demonstrated both by biopsy and high-resolution imaging.[9,16,27,29-32] Notably, the relative importance of modeling-based bone formation (MBF) in humans remains controversial.[5] In a recently reported study by *Dempster et al.*, however, it was reported that in women receiving teriparatide for 3 months, both modeling and remodeling-based formation (RBF) increased in the cancellous and endocortical compartments whereas only MBF increased in the periosteal envelope.[27] While the precise molecular mechanisms that underlie the net anabolic activity of PTH and PTH analogs are still being defined, they have been attributed to mechanisms both dependent and independent of bone remodeling, including:

1) Stimulating RANKL-mediated bone resorption, leading to the release of osteo-anabolic growth factors from the bone matrix.[33]
2) Suppressing expression of the Wnt inhibitor, sclerostin.[34]
3) Inhibition of osteoblast apoptosis.[35]
4) Activation of quiescent osteoblasts into bone forming osteoblasts.[20]
5) PTH induction of Wnt10b by activated T lymphocytes.[36]
6) Differentiation of bone marrow stromal cells (BMSCs) into cells of the osteoblast lineage.[37]

Effects of denosumab on bone metabolism and histomorphometry: Denosumab is a monoclonal antibody that inhibits the binding of RANKL to its receptor on cells of the osteoclastic lineage (RANK).[38] It is a potent antiresorptive agent that decreases both osteoclast and osteoblast activity within days of its administration and increases BMD while reducing fracture risk.[39-44] Biopsies taken from women treated with denosumab for up to 5 years have consistently demonstrated a decrease in bone resorption (eroded surface (ES)) as well as BFR and MS/BS. Moreover, up to 1/3 of patients treated with denosumab demonstrate an absence of either a double or single tetracycline label in trabecular and/or cortical compartments and 50% of patients show a complete absence of osteoclasts in the biopsy specimens.[27,39,41,45] *More recently, it was reported that in women who received denosumab for only 3-months, all types of bone formation were decreased or unchanged with the exception of modeling-based bone formation in the cancellous envelope, which increased significantly*.[46]

Effects of combination anabolic/antiresorptive therapy on bone metabolism and histomorphometry*:* While the RANK-ligand mediated mechanisms of bone resorption have been well studied over the past decade, the mechanisms of coupling of this bone resorption to bone formation are less well defined.[38,47] Potential coupling factors include matrix derived signals such as TGFβ, IGF-1,[48-51] secreted factors such as BMP-6 and Wnt10b,[47,52-54] as well as signals to and from other marrow components, including osteoclasts themselves.[55] Depending on the agents studied, animal models revealed variable findings on the effect of combined anabolic + antiresorptive agents on bone metabolism and histomorphometry.

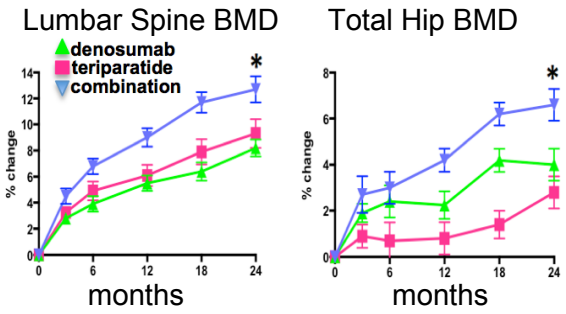
In the DATA study, the combination of denosumab and teriparatide resulted in less suppression of bone formation markers than seen with denosumab alone whereas bone resorption markers were potently and equally suppressed in both the denosumab alone and combination groups. Additionally, combining teriparatide and denosumab suppressed bone resorption markers more completely than has been reported in studies of combined teriparatide and bisphosphonates.[11,13,56] This likely contributes to the relatively greater increases in BMD with combined denosumab/teriparatide (discussed in detail below).

Quadruple Label Bone Biopsy in Humans: Paired iliac crest bone biopsies have classically been used to assess longitudinal changes in bone histomorphometry.[57] Quadruple labeling, however, with 2 pairs of tetracycline administrations separated by a period of weeks or months, permits longitudinal assessment with a single biopsy procedure.[58] Specifically, the mineral apposition rate and mineralizing perimeter can be measured for the two separate labeling periods, and changes in bone formation rates can thus be calculated at 2 separate time points. A further advantage of this technique is that each patient is able to serve as her own

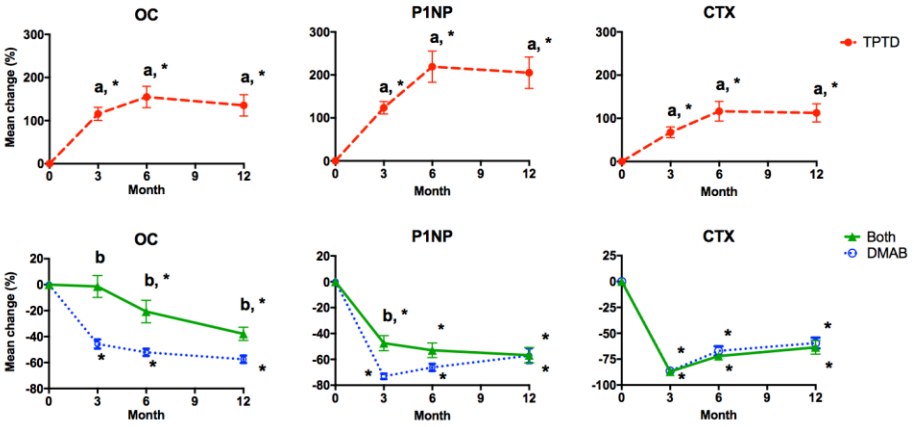
pre-treatment control, thus reducing variability in histomorphometric parameters and increasing statistical power at any given sample size. Indeed, quadruple-labeling has been used successfully to assess the effects of teriparatide and other osteoporosis medications in prior studies.[27] Moreover, this technique has proven especially useful in assessing the extent of modeling versus remodeling-based bone formation in the different bone envelopes,[27,58] and thus is a key tool to directly address the fundamental question regarding combination therapy that we are addressing in this proposal.

B. Preliminary Data:

The rationale and premise for this protocol comes directly from the data reviewed in the *Background/Significance* section above and the results of the DATA study. In DATA, postmenopausal women with osteoporosis were randomized to one of the following 2-year treatment groups: teriparatide 20-µg daily (n=31), denosumab 60-mg every 6 months (n=33), or the combination of both medications (n=30). Shown below are the 2-year changes in BMD in hip and spine *(P<0.05 vs other groups).



The changes in a biochemical markers of bone resorption (serum C-telopeptide, CTX) and bone formation (P1NP, osteocalcin) during the first year of treatment, the time during which the advantage of combination therapy is observed, are shown below.



[a] p<0·001 versus denosumab and versus combination; [b] p<0·01 versus denosumab; and *p<0·05 versus baseline. Bars show standard error of the mean. (Data for teriparatide group and other groups graphed separately for figure clarity).

These results demonstrate that unlike the combination of teriparatide and bisphosphonates, the combination of teriparatide and denosumab has additive effects on BMD. The mechanisms underlying these observations are the focus of this protocol.

Based on the results above, we hypothesize that that denosumab is able to fully block teriparatide's pro-resorptive effects (as indicated by the identical changes in CTX between the denosumab monotherapy and combined groups) while permitting ongoing modeling-based bone formation (as indicated by the early differences in osteocalcin and P1NP between the denosumab and combined groups). Confirmation of this hypothesis rerquires histomorphometric analysis as proposed in the current studies.

III. Specific Aims

The Specific Aim of this protocol is to test the following hypothesis:

Hypothesis: This study described below will tests the hypothesis that in postmenopausal osteoporotic women, combined PTH-receptor stimulation (teriparatide) and RANKL inhibition (denosumab) will result in histomorphometrically-demonstrated inhibition of teriparatide-induced skeletal remodeling while allowing for continued stimulation of modeling-based bone formation in all bone compartments (cancellous, endocortical, intra-cortical, and periosteal).

IV. Subject Selection

Study population: 36 female volunteers will be recruited in accord with institutional guidelines for clinical studies.

Participation will be based on meeting the following entry criteria:

Inclusion Criteria:
Must satisfy A and B and C below:
A. women aged ≥ 45
B. postmenopausal by either of the following criteria:
> 36 months since last spontaneous menses or
> 36 months since hysterectomy, plus serum FSH ≥ 40 units / liter if < 60 years
C. osteoporosis with high risk of fracture by one or more of the following criteria:

- DXA spine or hip T-score ≤ - 2.5;
- DXA spine or hip T-score ≤ - 2.0 plus ≥ 1 of the following BMD-independent risk factors for fracture: fracture after age 50, parental hip fracture after age 50, prior hyperthyroidism, inability to rise from a chair with one's arms elevated, current tobacco smoker.
- DXA spine, hip, or forearm T-score ≤ - 1.0 plus ≥ 1 adult low-trauma fracture (low-trauma fracture = fracture after no trauma; or fracture after falling ≤ 6 inches when stationary or moving slower than a run).

Exclusion Criteria: Exclusion criteria similar to those used for our prior osteoporosis studies using these medications will apply[11,13] (also see ClinicalTrials.gov #NCT00926380).

- confirmed serum alkaline phosphatase above upper normal limit with no explanation
- liver disease (AST or ALT > 2 x upper normal limit)
- renal disease (eGFR < 30mL/min)
- hypocalcemia or hypercalcemia (Ca <8.5 mg/dL or >10.5 mg/dL)
- abnormal blood PTH (intact PTH < 10 pg/mL or > 65 pg/mL)
- serum 25-OH vitamin D < 20 ng/ml or >60 ng/ml
- hematocrit < 32%
- history of malignancy (except non-melanoma skin carcinoma), radiation therapy, or gouty arthritis
- history of urolithiasis within the last one year
- significant cardiopulmonary disease including unstable coronary artery disease, stage D ACC/AHA heart failure or any other condition that the investigator deems may preclude the subject from participating safely or completing the protocol procedures
- major psychiatric disease that in the opinion of the investigator would preclude the subject from providing adequate informed consent or completing the protocol procedures
- excessive alcohol use or substance abuse that in the opinion of the investigator would preclude the subject from providing adequate informed consent or completing the protocol procedures
- known congenital or acquired bone disease other than osteoporosis (including osteomalacia, hyperparathyroidism, Paget's disease)
- history of intravenous bisphosphonate, strontium, denosumab, abaloparatide or teriparatide use

- history of oral bisphosphonate use within the last 5 years or if use >5 years ago, cumulative use > 1 year
- use within the past 3 months of estrogens, SERMs, or calcitonin
- use of oral or parenteral glucocorticoids for more than 14 days within the past 6 months
- known sensitivity to mammalian cell-derived drug products
- known sensitivity to teriparatide, denosumab, or any of their excipients
- tooth extraction or dental implant within the past 2 months or planned in the upcoming 2 months
- history of osteonecrosis of the jaw
- known sensitivity to tetracycline, demeclocycline or other antibiotics in this drug class
- continuous use of tetracycline for >1-month duration within the last 10 years

Source of subjects and recruitment methods
Recruitment flyers will be posted in approved locations throughout the MGH (including the MGH Bone Mineral Density Center) and email announcements will be sent through the Partners Clinical Research Program Network. Letters will be sent to subjects identified through RSVP for Health. Additionally, a mailing will be sent to targeted populations, including subjects who have expressed an interest in one of our research group's previous studies.

Subjects may be recruited from the clinical practice (MGH Endocrine Associates) of the principal investigator and co-investigator(s). To avoid potential coercion, after the investigator has briefly presented the study, the participant will be asked if they may be re-contacted for a more in-depth phone call from another study physician. Additionally, informed consent for screening will be completed by a research coordinator/study physician and informed consent for the main study will be completed by a study physician who is not a provider for each of the patients/subjects of this recruitment method.

NOTE: Entry criteria were established with input from the FDA (Reproductive Section). These criteria are similar to those used in our most recent human studies for which we have been able to meet all enrollment goals. Dr. Leder currently holds IND #122883 for the use of teriparatide in combination with denosumab. This protocol will be submitted to the FDA as an amendment.

V. Subject Enrollment
This outpatient study will either be conducted at one or more of the following sites: the General Clinical Research Center (GCRC), the Osteoporosis Research Center or the Endocrine Unit Clinical Space (all within the Massachusetts General Hospital.

The general design is a randomized, 3-arm, open label study.

Procedure for informed consent:
Screening only informed consent will be obtained by a member of the study staff at the Screen visit. Main study informed consent will be obtained by a study physician at the beginning of the first study visit.

Subjects will first be screened and those who appear eligible will have a medical history and interview with a study investigator at the first study visit and those who remain eligible will be randomized.

Groups Assignment and Randomization:
Those who remain eligible will be formally accepted into the study by a study investigator and assigned to one of the 3 interventions by computer-generated assignment using a randomly varying blocking scheme (block sizes of 3 or 6) to minimize the predictability of treatment assignments (1:1:1 ratio). To account for imbalance in the dropout rate as of August 11, 2021, group B will be closed. Subjects will be assigned to one of the 2 remaining interventions by computer-generated assignment using a randomly varying blocking schema (block size 2 or 4) to minimize the predictability of treatment assignments. Please see the Biostatistical analysis for re-analysis of power calculations.

Group A: daily subcutaneous teriparatide 20-µg (self-administered)
Group B: one dose of subcutaneous denosumab 60-mg (administered by the study physician).
Group C: daily subcutaneous teriparatide 20-µg + one dose of subcutaneous denosumab 60-mg

There will be 12 subjects in each group.

VI. Study Procedures

Telephone Screening and screening labs:
Members of research staff will screen all interested subjects over the telephone. If subjects meet initial criteria and are interested in the study, a member of the study staff will schedule a screening visit wherein the subject will sign a separate "screening only" informed consent form (ICF). This ICF will allow for a blood draw and bone mineral density testing.

Prior to signing this screening only ICF, subjects will be offered the option to speak with a physician investigator if they wish or if they have questions that can't be answered by the research assistant. Research assistants will document this conversation on study records.

At this screen visit, bone mineral density of the spine and hip will be obtained and the following will be measured in serum or blood:

- PTH, 25-OH vitamin D, uric acid, CBC, and comprehensive chemistry panel (serum glucose, BUN, Cr, eGFR, Na, K, Cl, Ca, total protein, albumin, bilirubin, ALP, ALT, AST)
- FSH (if necessary due to prior hysterectomy)

Participants who have serum uric acid levels > 6.5 mg/dL and are randomized to receive teriparatide will be treated with urate lowering therapy prior to receiving teriparatide.

If a subject's screen eligibility labs contain an abnormal result/s that, in the opinion of the investigator, should be repeated, the subject will be invited to have the abnormal test/s repeated with study staff or study staff will recommend that the subject have the test/s repeated with the subject's clinical care team.

If greater than 3 months have lapsed between the subject's screen labs and their first main study visit, study staff may request the subject to have all or some of their screening labs repeated for safety purposes.

If greater than 12 months have lapsed between the subject's screening visit and their first main study visit, study staff will invite subjects to undergo a re-screen for accuracy and safety purposes.

The study period lasts 3 months.

Visit 1:
If the subject meets the above BMD and laboratory criteria, she will then be scheduled for Visit #1. At this visit, a study physician will obtain written informed consent for participation in the complete study. If subjects require more time to consider participating after reading the main study ICF the study visit will not continue. If the subject is ready to sign the main study ICF, the study physician will then perform a history and physical on her. If all inclusion/exclusion criteria are met, subjects will then proceed with the rest of visit 1 and the subsequent visits.

Visit 1-4:
These visits will occur at the following times:
Visit 1 (pre-treatment, +/- 7 days)

Visit 2 (day 1, +/- 7 days)
Visit 3 (month 1, +/- 7 days)
Visit 4 (month 3, +/- 7 days)

Subjects will be seen at MGH prior to 10 a.m. and will be instructed to be fasting. The following blood/serum tests will be measured based on the schema and visit schedule below:

- Comprehensive chemistry panel (serum glucose, BUN, Cr, eGFR, Na, K, Cl, Ca, total protein, albumin, bilirubin, ALP, ALT, AST)
- Serum CTX (pooled* assay at end of study)
- Serum osteocalcin (pooled* assay at end of study)
- Serum PINP (pooled* assay at end of study)

*Samples for CTX, PINP and osteocalcin will be run in a batch assay at the conclusion of the study.

If a subject's visit labs contain an abnormal result/s that, in the opinion of the investigator, should be repeated, the subject will be invited to have the abnormal test/s repeated with study staff or study staff will recommend that the subject have the test/s repeated with the subject's clinical care team.

After which the subjects will be given breakfast, and then have the following procedures per table below:

Schema and visit schedule:

	Screen		**Visit 1**	**Visit 2 +7 days**	**Visit 3 +/- 7 days**	**Visit 4 +/- 7 days**
			Pre-treatment	**Day 1**	**Month 1**	**Month 3**
Informed Consent	**X**		**X**			
BMD by DXA (spine, hip)*	**X**					
Comprehensive chemistry panel	**X**			**X**	**X**	**X**
Uric acid, PTH, 25-OH vitamin D, CBC	**X**					
History and Physical Exam			**X**			
Randomization assignment			**X**			
Dispense labeling antibiotics with schedule			**X**		**X**	
Dispense teriparatide				**X**		
Administer denosumab				**X**		
Bone Turnover Markers (serum CTX, P1NP, osteocalcin)				**X**	**X**	**X**

*May use historic DXA performed for clinical indications or undergone as part of another protocol if the DXA scan is performed within 6 months of the Screen Visit.

At visit 2, denosumab (sold under the brand name Prolia) will be administered to subjects assigned to the appropriate group after all blood sampling is complete. Also at visit 2, subjects will be trained in the use of the teriparatide injection pen and will give themselves the first injection while observed by a member of the study staff. A sufficient supply of teriparatide (if assigned) will be given to each subject at the conclusion of visit 2. Fluorochrome labels will be administered as described in the section below.

All subjects who report dietary intake <1200mg of calcium daily will be given supplementation (600mg elemental calcium + 400 IU Vitamin D). Total calcium intake (dietary and supplemental) should be at most 1200mg/day. Subjects with a calcium dietary intake > 1200 mg will receive Vitamin D only. Participants who have a screen 25-OH vitamin D level > 50 ng/mL will refrain from vitamin D supplements for the first month and then start at 800 units/day.

Note: Any remaining serum samples will be saved and may be used to measure other variables related to bone metabolism at a future time. Samples will be stored with code number and date of sampling in a locked –80-degree freezer and only approved study personnel will have access to the samples. It is understood that any future use of samples must be reviewed and approved separately by the Partners Human Research Committee (PHRC) prior to their use. Personal-Identifying information will be removed before any sample is sent out for analysis to a non-Partners affiliated institution.

Note: The Principal Investigator may end the subject's participation without the subject's consent in order to protect the health of the subject, if the subject is unable to attend to study visits, if the sponsor decides to stop the study, or due to other administrative reasons.

Biochemical markers of bone turnovers of serum procollagen type I N-terminal propeptide (P1NP), serum osteocalcin, and serum β-c-terminal telopeptide of type one collagen (CTX) will be performed on fasting morning blood samples (24 hours after last injection if taking teriparatide). Samples will be run in batch assay at the conclusion of the study. Samples will be stored at -80C.

Iliac crest bone biopsy procedure:
Iliac crest bone biopsies will be performed at the 3-month time point, corresponding to the period of teriparatide's maximal stimulation of osteoblasts and anabolism.[11] The biopsies will be performed in the MGH Clinical Research Center by either Dr. Joseph Schwab, Dr. Stuart Hershman, Dr Daniel Tobert or Dr. Harold Fogel of MGH Orthopedic Surgery. A trained staff physician or nurse anesthetist will supervise anesthesia. Before proceeding, the procedure will be discussed with the participant in detail, including the risks involved (see human subjects section). A model (synthetic pelvic bone model) will be used to help explain the procedure with particular attention to the location of the biopsy. The use of short-acting oral (lorazepam/alprazolam) or parenteral sedation (midazolam and/or fentanyl, in titrated doses) will also be discussed with the participant and its use is optional depending upon the participant's comfort level. The iliac biopsy is obtained using a Bordier trephine, 7 mm in internal diameter, to avoid fracture and minimize sampling error. The biopsy site is 1 inch posterior to the antero-superior iliac spine and 1 inch inferior to the summit of the iliac crest. The skin and subcutaneous tissue will be injected with 1% xylocaine with epinephrine (1:~~2~~100,000) anesthetic (it is often necessary to re-dose during the procedure). The periosteum is then injected (both the superficial and the deep surface to prevent pain during biopsy). After the local anesthetic has been allowed to achieve its desired effect, the incision is made at the pre-marked site. The superficial surface of the ileum is identified and the trephine cutting surface is directly applied to the bone (the trephine will be marked with the desired depth of penetration in order to limit the depth of penetration into the iliac crest). The trephine is then turned in a clockwise fashion through the superficial cortical surface of bone and the cancellous bone (perpendicular with the iliac crest from the outer table to the inner table. The trephine is then removed and the biopsy specimen (core, including cancellous bone and a single cortical layer) is placed in 70% ethanol in a sterile container labeled with the subject's unique identifier. After similar local anesthesia, a second smaller core biopsy (3.5 mm x 2 cm) will be obtained from just posterior to the first biopsy. If the participant has provided consent, a bone marrow aspirate of 10 cc maximum will be obtained from the same site as the second biopsy. Adequacy of the aspirate will be confirmed by the presence of bony spicules. The 3.5 mm core will be homogenized in Qiazol and the aspirate will be filtered (100 micron mesh) and spun to separate cells from the marrow supernatant, the latter of which will be banked for future analyses of marrow cytokines/adipokines which is beyond the scope of the current proposal. Pressure is applied to ensure hemostasis and the biopsy site will be closed in layers using 3.0 monocryl sutures and the skin closed with

absorbable sutures. The procedure lasts 30-40 minutes. After the bone biopsy procedure, subjects will be instructed to lie flat for 2 hours in the Clinical Research Center before going home in order to minimize swelling and hematoma accumulation within the periosteum or adjacent muscles. Subjects will be instructed on strict bed rest for 24 hours after the procedure (apart from necessary activities such as eating/using the restroom) and then reduced activity for 14 days (no hiking/biking/exercise classes and walking less than 1 mile). As done by our collaborators at the Mayo Clinic, subjects will be instructed to use acetaminophen for analgesia. Tools used for the bone biopsy procedure will be sharpened and sterilized after each use.

Outcomes: **Note that the analysis of the biopsy specimens and other endpoints will be performed in a blinded fashion (i.e. the interpretation will be done without knowledge of group assignment).**

Bone Biopsy Microarchitectural and Histomorphometric Analyses will address the hypothesis that combined therapy with teriparatide and denosumab will inhibit teriparatide-induced skeletal remodeling while allowing for continued stimulation of modeling-based bone formation. Fluorochrome labels will be administered as reported by Dr. Dempster (see figure below, DEM=demeclocycline, TET=tetracycline).[58,59] The subject will receive 150 mg DEM QID for three days, no label for 12 days and DEM again for three days (3:12:3), ending 2-5 days prior to visit 1(baseline labeling). Tetracycline (TET, 250 mg) will be administered in a similar fashion prior to visit 3. The biopsy is performed 5–8 days following the last tetracycline dose.

Core biopsy samples will be fixed immediately in 70% ETOH and high-resolution micro-CT (μCT, μCT40, Scanco Medical AG) will be performed in the P30 Core facility to evaluate trabecular and cortical bone microarchitecture as previously published.[32,60-63]. The long axis of the biopsy will be aligned with the rotational axis of the scanner. Scans will be acquired with 70 kVP, 160 μAs with an integration time of 200 ms and nominal voxel size of 10 μm. Images will be reconstructed, subjected to Gaussian filtration and the mineralized tissue segmented using a global threshold. Trabecular and cortical morphometric indices will be assessed using direct 3D approaches.[64] Trabecular bone outcomes will include volumetric density, bone volume fraction, thickness, and separation, connectivity density and trabecular bone pattern factor. Cortical outcomes will include volumetric density, tissue mineral density, thickness and porosity.

The 8mm core biopsy specimen will then be embedded in methylmethacrylate. Dynamic parameters will be evaluated on unstained 10μm sections and static parameters will be evaluated on 5 micron sections stained with Massons Trichrome. Analyses will be performed on at least two nonconsecutive sections of each sample using Osteomeasure software. Mineralizing surface/bone surface will be calculated using the length of all double labels and the length of half of the single labels. Since demeclocycline can overestimate the length of the label relative to tetracycline, the length of the tetracycline labels will be multiplied by a correction factor of 1.34 as was done by our consultant Dr. Dempster.[59] Because each bone compartment has distinct remodeling characteristics, four compartments will be evaluated: cancellous (bone surround by bone marrow), endocortical (bone in contact with the inner boundary of the cortical bone), intracortical (Haversian canal surface), and periosteal (outer boundary of cortex). All parameters will be expressed according to the most recent guidelines from the ASBMR.[8] *Static Parameters* include osteoid surface, osteoid volume, osteoid thickness, osteoblast surface, osteoclast surface and eroded surface. In addition, the area occupied by marrow adipocytes (adipocyte volume/tissue volume) will be quantitated as will the number of osteoblasts, lining cells and osteoclasts (Nob/perimeter, NLc/ perimeter and NOcl/perimeter). *Dynamic parameters* include mineral apposition rate, mineralization surface, mineralization lag time, resorption period, resorption rate, and activation frequency. *Structural parameters* include trabecular bone area, trabecular thickness, trabecular number, trabecular separation, cortical width, osteocyte lacunar area and cortical porosity. Analyses will be performed according to Frost's method for tetracycline-labeled trabecular bone and the results will be interpreted with regard to Frost's "basic Multicellular unit" (BMU) theory.[65,66] One exception to BMU theory will

be made, however, since there is evidence that bone formation can occur without preceding resorption (modeling-based formation). A bone-forming unit will be identified by the presence of single or double tetracycline labels. Remodeling-based formation units (RmF.U) and modeling-based formation units (MF.U) in the unlabeled sections will be identified by examining the underlying reversal and cement lines in adjacent trichrome-stained sections. A scalloped reversal line indicates that the forming unit is remodeling-based, whereas a smooth cement line indicates that it is modeling-based. Furthermore, we will term a RmF.U with tetracycline label(s) extending beyond the extent of the scalloped reversal line unit as an overfilled remodeling unit (oRmF.U). Collagen fiber alignment will be evaluated under polarized microscopy to identify interrupted *vs.* uniformly oriented fibers, characteristic of remodeling and modeling-based formation respectively.

VII. Biostatistical Analysis

This study tests the hypothesis that in postmenopausal osteoporotic women, combined PTH-receptor stimulation (teriparatide) and RANKL inhibition (denosumab) will result in inhibition of teriparatide-induced skeletal remodeling while allowing for continued vigorous stimulation of modeling-based bone formation.

The primary endpoint of this study is the change from baseline to month 3 in the cancellous bone formation rate (BFR/BS) in order to test the hypothesis that modelling based bone formation is occurring in the combination therapy group.

Key secondary endpoints include MS/BS in the cancellous, endocortical, intracortical, and periosteal compartment separately as well as separate calculations of the proportion of the rates of remodeling-based bone formation (RBF/BS), modeling based bone formation (MBF/BS), and overflow MBF (oMBF) in the cancellous, endocortical, periosteal compartments.

Between-group differences in endpoints will be assessed by comparing each treatment group's 3-month mean change by one-way ANOVA followed by a post-hoc test. Normality of the outcome data will be examined by Shapiro-Wilk's test. If the outcomes are not normally distributed, a proper transformation will be performed before applying the ANOVA or the use of the Kruskal-Wallis test followed by Tukey Kramer parametric or Dunn's non-parametric post-hoc test.

All study participants who have had a bone biopsy at month 3 will be included in the primary outcome analysis. For the primary outcome analysis, we will adopt a Bonferroni corrected overall Type-1 error rate of 5% for the three-group comparisons (i.e., individual test's alpha = 0.017). For the secondary outcomes, the Type-1 error control for the multiple comparisons will not be considered (i.e., individual test's alpha = 0.05).

Additional secondary analyses will include an examination of the within-group mean change of each endpoint by paired samples t-test or Wilcoxon's signed rank test depending on the distribution of the outcome. The micro-CT analyses of human bone biopsy specimens are exploratory.

Effect size estimates: The power analyses were conducted using the estimated standard deviations reported in the AVA study (reference 46). This study utilized identical quadruple-labeling biopsy methodology in a short-term comparison of 2 different interventions (denosumab versus teriparatide). We conservatively predict that 10 subjects in each group will complete the study (83% completion rate). With this group size, the table below shows our effect size estimates for a 3-group multiple comparisons at a Bonferroni's corrected overall Type-1 error rate of 5% with 80% power. This detectable difference is much smaller than that reported in published studies comparing short term interventions in similar populations, including the AVA study in which BFR/BS increased from 0.0096 to 0.0366 $mm^3/mm^2/y$ (greater than 3-fold increase) in women treated with teriparatide and decreased from 0.0091 to 0.0014 $mm^3/mm^2/y$ (greater than 6-fold decrease) in women treated with denosumab. Similar differences were reported in endocortical MS/BS (increase from 9% to 38 with teriparatide and decrease from 9% to 7% with denosumab, between group difference >30%), intracortical MS/BS (increase from 9% to 21% with teriparatide and decrease from 10% to 3% with

denosumab, between group difference >19%), and periosteal MS/BS (increase from 1% to 4% with teriparatide and decrease from 1% to 0% with denosumab, between group difference >4%). Study participants that fall outside the month 3 study window will be included in the primary outcome analysis if the bone biopsy is performed within 21 days from the last tetracycline labeling dose AND if they continue to receive study drug throughout this time.

To estimate our ability to detect between-group differences in the changes in RBF/BS, MBF/BS, and oMBF/BS in cancellous, endocortical, and periosteal bone, we use the standard deviation from an unpublished analysis of the AVA study (Dempster, personal communication). The minimal detectable differences shown below are significantly smaller than the reported between group differences in the change of each parameter between denosumab and teriparatide treated women in the AVA study. For example, the difference between the 3-month change in cancellous MBF between teriparatide and denosumab treated women was 1.5% whereas we will be able to detect a difference of 0.15%. While differences in MBF between the combination group and either monotherapy group will likely be lower than those observed between teriparatide and denosumab, the study will still have sufficient power to detect small between-group differences. Thus, we will have adequate power to test our hypothesis that in postmenopausal osteoporotic women, treatment with combined teriparatide and denosumab will result in increased bone formation (compared to denosumab alone), and specifically modeling-based bone formation in all bone compartments.

The study will not have sufficient power to detect any differences in any safety endpoints between the three groups.

Parameter	Est. SD of the 3-month Δ	min detectable difference (5% sig, 80% power)
Cancellous BFR/BS ($mm^3/mm^2/y$)	0.005 ($mm^3/mm^2/y$)	0.007 ($mm^3/mm^2/y$)
Cancellous MS/BS (%)	2.60%	4.06%
Endocortical MS/BS(%)	5.11%	6.77%
Intracortical MS/BS(%)	7.57%	10.03%
Periosteal MS/BS(%)	1.32%	1.74%
Cancellous RBF (%)	3.06%	4.05%
Cancellous MBF (%)	0.11%	0.15%
Cancellous oMBF (%)	0.28%	0.37%
Endocortical RBF (%)	6.28%	8.32%
Endocortical MBF (%)	1.11%	1.47%
Endocortical oMBF (%)	0.89%	1.18%
Periosteal RBF (%)	0.44%	0.58%
Periosteal MBF (%)	3.11%	4.12%
Periosteal oMBF (%)	0.17%	0.23%

To account for imbalance in the dropout rate as of August 11, 2021, the following power calculation analysis is for an outcome of predicted 8 subjects in each group. Effect sizes were estimated for a 3-group multiple comparisons at a Bonferroni's corrected overall Type-I error rate of 5% (alpha 0.017) with 80% power. For the 8-8-8 outcomes, the detectable differences for our primary endpoint as well as a number of key secondary endpoints is much smaller than that reported in published studies comparing short term interventions in similar populations, in particular the AVA study (reference 46). While between-group differences between the combination group and either monotherapy group will be smaller than the between-group differences between teriparatide and denosumab, the study will have sufficient power to detect small between-group differences.

Primary Endpoint:

1. Cancellous BFR/BS, minimum detectable difference for a 8-8-8 outcome is 0.009 mm3/mm2/y. Between group difference reported in the AVA study (denosumab vs. teriparatide) was 0.0347 mm3/mm2/y.

Secondary Endpoints:
1. Cancellous MS/BS = 4.65 for a 8-8-8 outcome, between group difference in AVA study = 17.33
2. Endocortical MS/BS = 9.14 for a 8-8-8 outcome, between group difference in AVA study = 33.89
3. Intracortical MS/BS = 13.54 for a 8-8-8 outcome, between group difference in AVA study = 19.81
4. Periosteal MS/BS = 2.36 for a 8-8-8 outcome, between group difference in AVA study = 4.44
5. Cancellous RBF = 5.47 for a 8-8-8 outcome, between group difference in AVA study = 12.718
6. Cancellous MBF = 0.20 for a 8-8-8 outcome, between group difference in AVA study = 1.244

VIII. Risk and Discomforts
Risks of these medications are summarized below:

Denosumab
DMAB is an FDA approved medication used to treat osteoporosis in various populations, including postmenopausal women. More than 13,500 patients have been treated with denosumab in clinical studies, and it is generally well tolerated.[6,43,67] In clinical studies, it has been reported that DMAB may produce the following side-effects: hypersensitivity including anaphylactic reaction, hypocalcemia, osteonecrosis of the jaw, atypical femoral fractures, multiple vertebral fractures following denosumab discontinuation, serious infections including skin infections and cystitis, dermatitis, rashes, eczema, musculoskeletal pain, and hypercholesterolemia. Pancreatitis has been reported in clinical trials. To reduce the likelihood of adverse events, subjects with any contraindications to denosumab, including dental procedures, will be excluded. Subjects will be monitored for report of thigh or groin pain and would be evaluated to rule out an atypical femur fracture. Subjects who withdraw consent during the study will be counseled on the increased risk of fracture when denosumab is discontinued and not followed by subsequent osteoporosis treatment and offered a referral to a specialist if desired.

Teriparatide
TPTD is an FDA approved medication used to treat postmenopausal women with osteoporosis who are at high risk of fracture. The most commonly reported side effects of TPTD are overall pain, asthenia, nausea, headache, leg cramps, sinus tachycardia, arthralgia, rhinitis, and dizziness. TPTD can cause transient hypercalcemia, which is generally mild.[7] Repeated daily administration of TPTD in high doses to rodents causes dose-dependent osteosclerosis, bony obliteration of the marrow space, and extra-medullary hematopoiesis, followed by osteosarcomas.[68] The relevance of these findings to humans is unclear and will remain so for many years, because osteosarcoma is rare in adults (4 cases/million/year).[69] Eli Lilly and the FDA are monitoring the incidence of osteosarcoma in TPTD treated patients and have not reported a linkage thus far. Hypercalcemia is a well-described complication of TPTD treatment. Elevated blood calcium levels will be handled in the manner recommended by Antoniucci et al.[5] (Figure 1)

Antibiotic Labels: Short-term administrations of the *tetracycline and demeclocycline* are generally very well tolerated with the most common side effect being gastrointestinal symptoms such as anorexia, nausea, and vomiting. Rarely, maculopapular and erythematous rashes, erythema multiforme and Stevens-Johnson syndrome have been reported. Hypersensitivity reactions including urticaria, angioneurotic edema, polyarthralgia, and anaphylaxis have also been rarely reported.

Sedation with Lorazepam/Alprazolam/Midazolam/Fentanyl (optional):
Subjects may opt to have sedation during the bone biopsy procedure. Sedation is generally very safe but there is still a risk of side effects. If these side effects do occur, the majority of them are temporary, although long-term complications may occur. All reasonable precautions will be applied to avoid any complications. Common

side effects include nausea and vomiting, headache, sore or dry throat, dizziness or feeling faint, fall in blood pressure or a mild allergic reaction such as itching or a rash. If the level of sedation is too deep, subjects may experience some difficulty breathing that may require breathing support until the effects of the sedation wear off. In rare cases, there can be serious allergic reactions or cardiac arrest (stopping of the heart).

Ondansetron (optional):
If subjects experience nausea or vomiting from the sedation during the bone biopsy procedure, they will be offered ondansetron to help minimize these side effects. Common side effects of ondansetron include headache, dizziness, drowsiness, constipation or diarrhea. Additional rare side effects include serious arrythmias and hypoxia.

Risks of Bone Biopsy Procedure:
Bone biopsies of the iliac crest are associated with discomfort but pain can be largely eliminated or reduced when the skin and periosteum are adequately treated with local anesthetic prior to the procedure. Serious complications are very rare but include pain, hematoma, and wound infection. Neuropathy (transient) is an extremely unusual complication of bone biopsies. In the most comprehensive study of bone biopsy complications observed in 9131 trans-iliac biopsies, the incidence of overall complications was 0.6%; 0.2% experienced prolonged pain, 0.2% developed a hematoma, 0.12% experienced transient neuropathy, and 0.07% developed a wound infection. Two patients (0.02%) experienced hip fracture, and one patient (0.01%) developed osteomyelitis. No patient died or experienced permanent disability.[70] The short courses of tetracycline and demeclocycline antibiotics are generally well tolerated but can cause gastrointestinal discomfort and sun-sensitivity as discussed above.

Minimizing biopsy risks:
As stated above, the common risks of the procedure are discussed in detail with the participant. The signs and symptoms of infection (fever above 101.5, a draining wound, expanding redness around the incision, worsening pain and chills) are described and the subject is instructed to contact study staff immediately should any of these occur. Post-procedural pain is a risk of the procedure although not technically a complication. The subject is instructed that ice be applied to the biopsy site using a cloth barrier between the ice pack and the subject's skin. The subject is also instructed that over the counter pain medications should also be used to help manage pain (acetaminophen and non-steroidal anti-inflammatory medicine). Thigh numbness and pain are uncommon complications of this procedure caused by damage to the lateral branch of the lateral femoral cutaneous nerve (LFCN). The biopsy is taken 3 cm lateral to the anteriorsuperior iliac spine to avoid the lateral branch of the LFCN. Some bleeding after the biopsy is expected. It is explained to the subject that while some bloody staining of the dressing applied at the end of the procedure is to be expected; bloody drainage from the incision should be reported to the investigator immediately. Due to the anesthesia, subjects will be instructed to arrange for transportation from the office to their home. Subjects will be asked to limit physical activity and hard physical work 3-4 days after the biopsy and not to exercise for 7 days after the biopsy.

All adverse events will be reported to the IRB either immediately (in the case of definitely related or probably/possibly related serious adverse events) or at the time of the scheduled renewal process (for all other adverse events). The study physicians and the study staff will meet on a weekly basis to discuss any issues relating to adverse events, safety data and outcome data. The risks of bone biopsy will be minimized by careful attention to proper technique and will be performed by a board-certified orthopedic surgeon.

Imaging studies
Over the 3-month study period, subjects will receive only a single DXA of the spine and hip, with consequent radiation of <1 mrem. This amount of radiation is equal to the background radiation one is exposed from the earth and sky under one month.

IX. Potential Benefits
The medications given in this study have been shown to reduce fracture risk in postmenopausal women with

osteoporosis when used according to their FDA approved label; however, it is unknown if subjects' osteoporosis will get better, worse, or stay the same in this study. It is hoped that the knowledge gained from this study will improve osteoporosis care. Given the short-term nature of the study, participants will be encouraged to discuss continuing osteoporosis care with their providers at the end of the study.

Subjects will be compensated for their time with $25 after each visit and $400 for completing the bone biopsy, for a total of $500. Subjects will not be compensated for the screen visit and will be mailed a copy of their screen visit bone density results.

Parking vouchers will be provided to subjects at each visit, including the screening visit.

X. Monitoring and Quality Assurance

The Institutional Review Board (IRB) will review and approve the study protocol, ICF and any other applicable subject material before any study-specific activities or procedures begin. Before a subject can participate in any study-specific activities/procedures, she will sign, or have a legally authorized representative sign and date the IRB-approved ICF.

A study physician will review all laboratory test results. The study physicians and the study staff meet on a weekly basis to discuss any issues relating to adverse events, safety data and outcome data. Subjects will be discontinued from the study if they have any finding that in the opinion of the study physician requires withdrawal from the study. Disqualifying findings will be communicated to both the subject and her primary care physician. If the subject does not have a primary care physician, the principal investigator will coordinate appropriate follow-up with a physician of the subject's choosing.

Study Completion and Closeout Procedures

Dr. Leder and Dr. Tsai will verify that:
- study interventions are complete and that all data have been collected
- correspondence and study files are accessible for audits
- study records are maintained
- NIAMS and the IRB are notified of the study's completion and store a copy of the notifications
- a clinicaltrials.gov report is completed
- subjects are notified of study completion and asked if they would like to be informed of the results and/or receive a copy of the publication of the trial results

At their third study visit, all study participants will meet with a study physician. The study physician will review the experience of the participant during the study and explain to the participant that it is imperative that they arrange follow up with their primary care physician within 3 months of finishing the study, due to the increased risk of multiple vertebral fractures when denosumab is discontinued and not followed by subsequent osteoporosis treatment. Subjects will be encouraged to see a physician with some expertise in the treatment of osteoporosis and offered the opportunity to schedule an appointment in the Endocrine Associates outpatient clinic to discuss therapy beyond the study period. With the subject's permission, a letter will be sent to their primary care physician, at completion of the study, including this information and the results of all relevant investigations from the study period. Study participants will be reminded of this information at their final study visit and a follow-up phone call will be made 3 months after exit from the study to ensure that they have received appropriate follow-up.

The Data Safety Monitoring Board (DSMB) will monitor this study. This DSMB is comprised of experienced clinical investigators with expertise in osteoporosis: Dr. Kristine Ensrud, Dr. Susan Ott, and Dr. George Howard.

<u>Definition of Adverse Events</u>

An adverse event (AE) is any unfavorable and unintended diagnosis, sign (including an abnormal laboratory

finding), symptom, or disease temporarily associated with the study intervention, which may or may not be related to the intervention. AEs include any new events not present during the pre-intervention period or events that were present during the pre-intervention period which increased in severity.

Definition of Serious Adverse Event
A serious adverse event (SAE) is any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or, in the opinion of the investigators, represents other significant hazards or potentially serious harm to research participants or others.

Definition of Unanticipated Problems
Unanticipated problems (UPs) are defined as being unexpected in terms of nature, severity, or frequency based on the IRB-approved research protocol, informed consent documents and the participant population being studied; being related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the UP may have been caused by the procedures involved in the research); AND suggesting that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Classification of an Adverse Event
All AEs will be assessed by the study clinician and assigned to the following categories:

Mild: Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate: Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Relationship to Study Intervention
All AEs will have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definite: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probable/Possible: There is evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication), but other factors are present which may have contributed to the event (e.g. the participant's clinical condition, other concomitant events). However, there is greater than 50% certainty that the event is related to study intervention. The event follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition. Although an AE may rate as "probable/possible" upon discovery, it can be flagged as requiring more information and later be upgraded to "definite," as appropriate.

Unrelated/Unlikely related: The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely or likely related to another etiology.

Expectedness
The study investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Time Period and Frequency for Event Assessment and Follow-Up
The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

A Study Team member will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation, unless reporting is deemed necessary in the opinion of the study investigator. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Reporting of Adverse Events
Adverse events and serious adverse events will be reported to all appropriate regulatory bodies as required by current regulations. Study staff will record all adverse events in the Adverse Event Log upon learning of such events. The Adverse Event Log will be shared annually with the IRB at the time of Continuing Review and with KAI at the time of the annual meeting. Study staff will also complete a Serious Adverse Event Form for all serious adverse events, to be filed in the subject's study file. After identification of any adverse event, the PI will advise the study team regarding screening, enrollment, and ongoing participation and initiatives to prevent further AEs if relevant. The principal investigator or Dr. Demay or Dr. Tsai will evaluate the adverse event and determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol or consent form are required. The PI will be responsible for determining if the study protocol may be continued, terminated, or modified based on the observed beneficial or adverse effects of the treatment under study.

Reports submitted to Amgen will include an annual and final safety report plus any other aggregate analyses (of any report containing safety data generated during the course of the study) submitted to the Partners IRB.

Adverse Events Requiring Immediate Reporting
Serious adverse events
- Study staff will notify KAI of all serious adverse events within 48 hours of learning of such events. KAI will notify the NIAMS and the DSMB. This will be followed by a written report as soon as possible.
- Study staff will notify the FDA of all serious adverse events within 15 calendar days. The sponsor of the IND (Dr. Leder) will adjudicate all adverse events in determining whether reporting to the FDA is necessary. In the

case that such an event causes death or is life-threatening, this will be reported to the FDA within 7 calendar days. An event is considered life-threatening, as defined by the FDA, if "in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death" (21 CFR 312.32). Non-serious adverse events do not require reporting to the FDA.
- If a serious adverse event is determined to be definitely or probably/possibly related to denosumab, a report of the event will also be submitted to Amgen at the same time as the IRB report.

Unexpected adverse events (serious or non-serious) that are definitely related or probably/possibly related
- Study staff will report the event to the IRB within 5 business days or 7 calendar days

Reporting of Unanticipated Problems
The following procedures will be followed for all Unanticipated Problems (UPs) that do *not* meet the definition of an adverse event.

Unanticipated problems will be reported within 48 hours to KAI (followed by a written report) and within 7 calendar days to the IRB. Unanticipated problems that do not meet the definition of an adverse event will be reported to the IRB as an 'Other Event' submission on Insight, and must include the following information: a detailed description of the unanticipated problem; the basis for determining that the problem is unexpected; the basis for determining that the problem indicates that the research places subjects at an increased risk of harm; and whether any changes to the research or other corrective action are warranted. The PI will advise the study team regarding screening, enrollment, and ongoing participation after identification and resolution of these events.

Further clarification on which events require immediate reporting can be found in the Partners Healthcare policy on Reporting Unanticipated Problems including Adverse Events (for reporting to the IRB) and 21 CFR 312.32 (for reporting to the FDA).

All subjects will be informed of their rights under the Health Insurance and Portability and Accountability Act of 1996 per federal law and hospital policy. The screening only and main study ICFs contain an IRB-approved template that describes what protected health information from the research study may be used or shared with others. Data collected strictly for research purposes are stored in locked files and on computers with passwords required for access. When the data are published, no names or other materials that allow identification of an individual will be used.

The study database will be secured with password protection and only IRB-approved study staff will have access. The study biostatistician will receive only de-identified coded information. Electronic or written communication with any outside collaborators will involve only unidentifiable information. Adverse event reports and annual continuing reviews will not include subject identifiable material. Data will be entered into REDCap, a free, secure, web-based application designed to support data entry for research studies. REDCap was developed by a multi-institutional consortium initiated at Vanderbilt University. Data collection will be customized for this project by the research team with guidance from Harvard Catalyst staff. The REDCap built-in system for data verification to safeguard against incorrect data entry will be used (e.g. data entry to be performed twice and verified within REDCap). REDCap is designed to comply with HIPAA regulations. Data within REDCap will be categorized as verified or unverified. Once all verified data are acquired and entered, the final dataset will be locked within REDCap. De-identified information sets may be extracted from REDCap to be shared with outside collaborators as needed, which would be performed only in accordance with the Partners IRB approval and the ICF. Collaborators will be provided with de-identified, secure data to be used solely for research. Collaborators will be requested to return or destroy data once data analysis is complete.

XI. Publication Policy
Publication of complete data from the study is planned. It is anticipated that the results of

this study will be published in a peer reviewed scientific or medical journal and may be presented at scientific meetings. Investigators will publish results from the study in compliance with their agreement with Amgen.

XII. Abbreviations

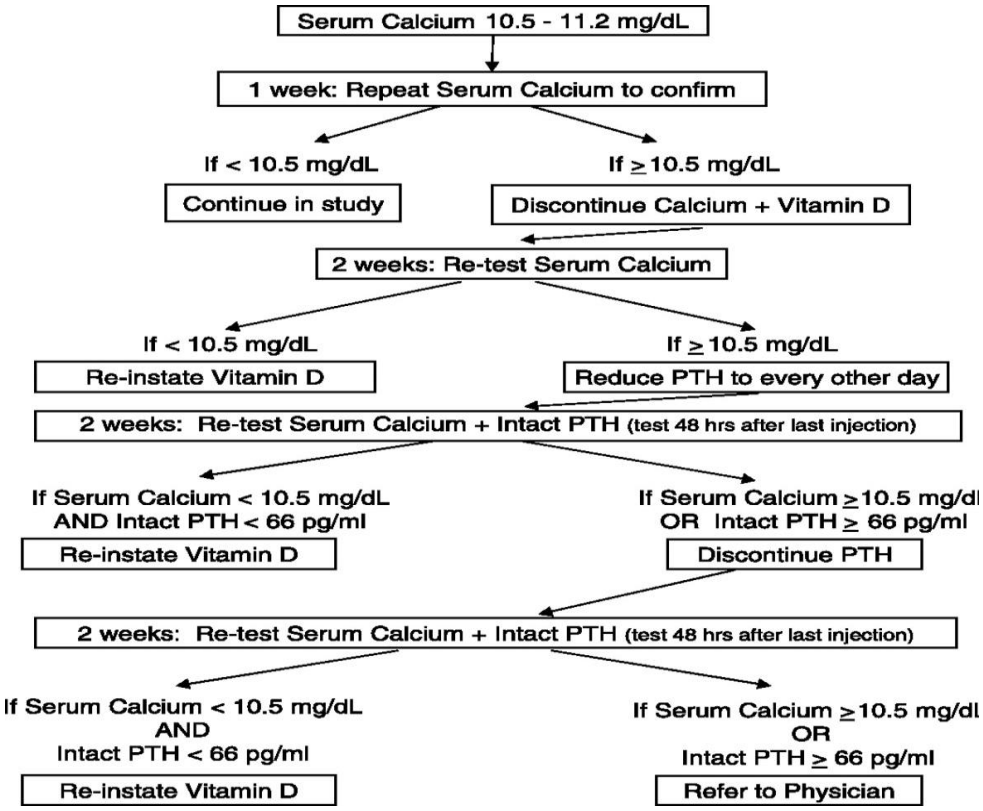
25-OH vitamin D	25-Hydroxy Vitamin D
ACC	American College of Cardiology
AE	Adverse Event
AHA	American Heart Association
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
ASBMR	American Society for Bone and Mineral Research
AST	Aspartate Aminotransferase
BFR	Bone Formation Rate
BMD	Bone Mineral Density
BMP6	Bone Morphogenetic Protein 6
BMSCs	Bone Marrow Stromal Cells
BS	Bone Surface
BUN	Blood Urea Nitrogen
Ca	Calcium
CBC	Complete Blood Count
CFR	Code of Federal Regulations
Cr	Creatinine
CT	Computed Tomography
CTX	C-Telopeptide
DATA	Denosumab and Teriparatide Administration
DEM	Demeclocycline
DSMB	Data Safety Monitoring Board
DXA	Dual-Energy X-Ray Absorptiometry
ES	Eroded Surface
ETOH	Ethanol
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GCRC	General Clinical Research Centre
PHRC	Partners Human Research Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IGF-1	Insulin Like Growth Factor 1
IND	Investigational New Drug Application
IRB	Institutional Review Board
LFCN	Lateral Femoral Cutaneous Nerve
MBF	Modeling-Based Bone Formation
MF.U	Modeling-Based Formation Units
MGH	Massachusetts General Hospital
MS	Mineralizing Surface
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases
oMBF	Overflow Modeling Based Bone Formation
oRmF.U	Overfilled Remodeling Unit
P1NP	Procollagen Type 1 N-Terminal Propeptide
Phos	Phosphate
PTH	Parathyroid Hormone

PTHrP	Parathyroid Hormone-Related Peptide
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RBF	Remodeling-Based Formation
RmF.U	Remodeling-Based Formation Units
SAE	Serious Adverse Event
SD	Standard Deviation
SERMs	Selective Estrogen Receptor Modulators
TET	Tetracycline
TGF-ß	Transforming Growth Factor-Beta
TPTD	Teriparatide
UPs	Unanticipated Problems
US	United States

XIII. Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
2.0	08 Feb 2019	Page 7: blood samples will be stored by code number and date only, not participant name	To ensure de-identification of study participants
3.0	27 June 2019	Changes made in response to KAI and DSMB document review and teleconference on 04/18/2019 and 06/27/2019.	Changes made in response to KAI and DSMB document review and teleconference on 04/18/2019 and 06/27/2019.
4.0	16 July 2019	Changes made in respond to KAI and DSMB document review following 6/27/19 teleconference.	Changes made in respond to KAI and DSMB document review following 6/27/19 teleconference.
5.0	26 November 2019	FSH testing only required if women are <60 years; change of oral BP exclusion criteria; addition of 3 other orthopedic staff who will perform the bone biopsies; addition of short-acting oral sedation to list of sedative meds that can be given during the bone biopsy.	
6.0	05 May 2020	Modifications to safety reporting procedures.	Language was clarified to match IRB and FDA policies.
7.0	01 April 2021; revised June 01 2021	Changes made to allow for labs/DXA to be repeated under certain circumstances and to allow for DXA's done for clinical indication or under other study protocols to be used for this study if done within 6 months of screen date. Adverse event reporting language clarified. Risk of sedation and ondansetron (optional meds) previously included in main ICF have been included in the protocol. Changes made on June 01, 2021 to reflect feedback received from expert external reviewer, Dr. Paul Miller, in context of adverse events (OE 11 and OE 12).	Changes made in response to QI audit, and recent subject adverse event.
8.0	08 July 2021	Change made to collect 10cc bone marrow instead of maximum 40cc	Change made in response to suggestion made by our NIH-appointed DSMB.
9.0	11 August 2021	Change made to close assignment to the denosumab only group and to state clearly how the change in randomization assignment will occur moving forward.	Change made in response to an imbalance in the study dropout rate amongst the 3 study groups and in response to the research pharmacy's request for a revision in detailed protocol.

Figure 1: Hypercalcemia workflow



If the serum calcium concentration rises above 11.2 mg/dl, TPTD will be stopped and serum calcium measured at least daily until normal. Thereafter TPTD will be resumed every other day and the above algorithm followed, starting at "Reduce PTH to every other day".

REFERENCES

1. Bone health and osteoporosis: A report of the Surgeon General. In: United States Department of Health and Human Services PHS, ed.: Office of the Surgeon General; 2004.
2. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet 2002;359:1761-7.
3. Bliuc D, Nguyen ND, Milch VE, Nguyen TV, Eisman JA, Center JR. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. Jama 2009;301:513-21.
4. Black DM, Cummings SR, Karpf DB, et al. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet 1996;348:1535-41.
5. Antoniucci DM, Sellmeyer DE, Bilezikian JP, et al. Elevations in serum and urinary calcium with parathyroid hormone (1-84) with and without alendronate for osteoporosis. J Clin Endocrinol Metab 2007;92:942-7.
6. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 2009;361:756-65.

7. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 2001;344:1434-41.
8. Shane E, Burr D, Abrahamsen B, et al. Atypical subtrochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res 2014;29:1-23.
9. Balasubramanian A, Tosi LL, Lane JM, Dirschl DR, Ho PR, O'Malley CD. Declining rates of osteoporosis management following fragility fractures in the U.S., 2000 through 2009. J Bone Joint Surg Am 2014;96:e52.
10. Imaz I, Zegarra P, Gonzalez-Enriquez J, Rubio B, Alcazar R, Amate JM. Poor bisphosphonate adherence for treatment of osteoporosis increases fracture risk: systematic review and meta-analysis. Osteoporos Int 2010;21:1943-51.
11. Finkelstein JS, Wyland JJ, Lee H, Neer RM. Effects of teriparatide, alendronate, or both in women with postmenopausal osteoporosis. J Clin Endocrinol Metab 2010;95:1838-45.
12. Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM. The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. N Engl J Med 2003;349:1216-26.
13. Finkelstein JS, Leder BZ, Burnett SM, et al. Effects of teriparatide, alendronate, or both on bone turnover in osteoporotic men. J Clin Endocrinol Metab 2006;91:2882-7.
14. Cosman F, Eriksen EF, Recknor C, et al. Effects of intravenous zoledronic acid plus subcutaneous teriparatide [rhPTH(1-34)] in postmenopausal osteoporosis. J Bone Miner Res 2011;26:503-11.
15. Leder BZ, Tsai JN, Uihlein AV, et al. Two years of Denosumab and teriparatide administration in postmenopausal women with osteoporosis (The DATA Extension Study): a randomized controlled trial. J Clin Endocrinol Metab 2014;99:1694-700.
16. Leder BZ, Tsai JN, Neer RM, Uihlein AV, Wallace PM, Burnett-Bowie SM. Response to Therapy With Teriparatide, Denosumab, or Both in Postmenopausal Women in the DATA (Denosumab and Teriparatide Administration) Study Randomized Controlled Trial. J Clin Densitom 2016.
17. Frolik CA, Black EC, Cain RL, et al. Anabolic and catabolic bone effects of human parathyroid hormone (1-34) are predicted by duration of hormone exposure. Bone 2003;33:372-9.
18. Uihlein AV, Leder BZ. Anabolic therapies for osteoporosis. Endocrinol Metab Clin North Am 2012;41:507-25.
19. Silva BC, Bilezikian JP. Parathyroid hormone: anabolic and catabolic actions on the skeleton. Curr Opin Pharmacol 2015;22:41-50.
20. Kim SW, Pajevic PD, Selig M, et al. Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts. J Bone Miner Res 2012;27:2075-84.
21. Lindsay R, Zhou H, Cosman F, Nieves J, Dempster DW, Hodsman AB. Effects of a one-month treatment with PTH(1-34) on bone formation on cancellous, endocortical, and periosteal surfaces of the human ilium. J Bone Miner Res 2007;22:495-502.
22. Ma YL, Zeng Q, Donley DW, et al. Teriparatide increases bone formation in modeling and remodeling osteons and enhances IGF-II immunoreactivity in postmenopausal women with osteoporosis. J Bone Miner Res 2006;21:855-64.
23. Delmas PD, Licata AA, Reginster JY, et al. Fracture risk reduction during treatment with teriparatide is independent of pretreatment bone turnover. Bone 2006;39:237-43.
24. Paschalis EP, Glass EV, Donley DW, Eriksen EF. Bone mineral and collagen quality in iliac crest biopsies of patients given teriparatide: new results from the fracture prevention trial. J Clin Endocrinol Metab 2005;90:4644-9.
25. Chen P, Satterwhite JH, Licata AA, et al. Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. J Bone Miner Res 2005;20:962-70.

26. Lindsay R, Scheele WH, Neer R, et al. Sustained vertebral fracture risk reduction after withdrawal of teriparatide in postmenopausal women with osteoporosis. Arch Intern Med 2004;164:2024-30.
27. Cosman F, Dempster DW, Nieves JW, et al. Effect of Teriparatide on Bone Formation in the Human Femoral Neck. J Clin Endocrinol Metab 2016:jc20153698.
28. Dempster DW, Zhou H, Recker RR, et al. Skeletal histomorphometry in subjects on teriparatide or zoledronic acid therapy (SHOTZ) study: a randomized controlled trial. J Clin Endocrinol Metab 2012;97:2799-808.
29. Hansen S, Hauge EM, Beck Jensen JE, Brixen K. Differing effects of PTH 1-34, PTH 1-84, and zoledronic acid on bone microarchitecture and estimated strength in postmenopausal women with osteoporosis: an 18-month open-labeled observational study using HR-pQCT. J Bone Miner Res 2013;28:736-45.
30. Portero-Muzy NR, Chavassieux PM, Bouxsein ML, Gineyts E, Garnero P, Chapurlat RD. Early effects of zoledronic acid and teriparatide on bone microarchitecture, remodeling and collagen crosslinks: Comparison between iliac crest and lumbar vertebra in ewes. Bone 2012;51:714-9.
31. Macdonald HM, Nishiyama KK, Hanley DA, Boyd SK. Changes in trabecular and cortical bone microarchitecture at peripheral sites associated with 18 months of teriparatide therapy in postmenopausal women with osteoporosis. Osteoporos Int 2011;22:357-62.
32. Recker RR, Bare SP, Smith SY, et al. Cancellous and cortical bone architecture and turnover at the iliac crest of postmenopausal osteoporotic women treated with parathyroid hormone 1-84. Bone 2009;44:113-9.
33. Bollerslev J, Harris ST, Leder BZ. Glucocorticoid-induced osteoporosis. J Clin Endocrinol Metab 2012;97:35A.
34. Keller H, Kneissel M. SOST is a target gene for PTH in bone. Bone 2005;37:148-58.
35. Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. J Clin Invest 1999;104:439-46.
36. Li F, Sun X, Ma J, et al. Naringin prevents ovariectomy-induced osteoporosis and promotes osteoclasts apoptosis through the mitochondria-mediated apoptosis pathway. Biochem Biophys Res Commun 2014.
37. Fan Y, Hanai JI, Le PT, et al. Parathyroid Hormone Directs Bone Marrow Mesenchymal Cell Fate. Cell Metab 2017;25:661-72.
38. Lacey DL, Boyle WJ, Simonet WS, et al. Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. Nature reviews Drug discovery 2012;11:401-19.
39. Eastell R, Christiansen C, Grauer A, et al. Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. J Bone Miner Res 2011;26:530-7.
40. 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.
41. Brown JP, Reid IR, Wagman RB, et al. Effects of up to 5 Years of Denosumab Treatment on Bone Histology and Histomorphometry: The FREEDOM Study Extension. J Bone Miner Res 2014.
42. 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.
43. 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.
44. Bolognese MA, Teglbjaerg CS, Zanchetta JR, et al. Denosumab Significantly Increases DXA BMD at Both Trabecular and Cortical Sites: Results From the FREEDOM Study. J Clin Densitom 2012.
45. Brown JP, Dempster DW, Ding B, et al. Bone remodeling in postmenopausal women who discontinued denosumab treatment: off-treatment biopsy study. J Bone Miner Res 2011;26:2737-44.
46. Dempster DW, Zhou H, Recker RR, et al. Remodeling- and Modeling-Based Bone Formation With Teriparatide Versus Denosumab: A Longitudinal Analysis From Baseline to 3 Months in the AVA Study. J Bone Miner Res 2018;33:298-306.
47. Baron R, Rawadi G. Wnt signaling and the regulation of bone mass. Curr Osteoporos Rep 2007;5:73-80.

48. Howard GA, Bottemiller BL, Turner RT, Rader JI, Baylink DJ. Parathyroid hormone stimulates bone formation and resorption in organ culture: evidence for a coupling mechanism. Proc Natl Acad Sci U S A 1981;78:3204-8.
49. Tang Y, Wu X, Lei W, et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 2009;15:757-65.
50. Yee JA, Yan L, Dominguez JC, Allan EH, Martin TJ. Plasminogen-dependent activation of latent transforming growth factor beta (TGF beta) by growing cultures of osteoblast-like cells. J Cell Physiol 1993;157:528-34.
51. Campbell PG, Novak JF, Yanosick TB, McMaster JH. Involvement of the plasmin system in dissociation of the insulin-like growth factor-binding protein complex. Endocrinology 1992;130:1401-12.
52. Sims NA, Jenkins BJ, Quinn JM, et al. Glycoprotein 130 regulates bone turnover and bone size by distinct downstream signaling pathways. J Clin Invest 2004;113:379-89.
53. Walker EC, McGregor NE, Poulton IJ, et al. Cardiotrophin-1 is an osteoclast-derived stimulus of bone formation required for normal bone remodeling. J Bone Miner Res 2008;23:2025-32.
54. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U S A 2008;105:20764-9.
55. Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. BoneKEy reports 2014;3:481.
56. Black DM, Greenspan SL, Ensrud KE, et al. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Engl J Med 2003;349:1207-15.
57. Compston JE. Histomorphometric interpretation of bone biopsies for the evaluation of osteoporosis treatment. BoneKEy reports 2012;1:47.
58. Lindsay R, Cosman F, Zhou H, et al. A novel tetracycline labeling schedule for longitudinal evaluation of the short-term effects of anabolic therapy with a single iliac crest bone biopsy: early actions of teriparatide. J Bone Miner Res 2006;21:366-73.
59. Dempster DW, Zhou H, Recker RR, et al. Differential Effects of Teriparatide and Denosumab on Intact PTH and Bone Formation Indices: AVA Osteoporosis Study. J Clin Endocrinol Metab 2016;101:1353-63.
60. Miki T, Nakatsuka K, Naka H, et al. Effect and safety of intermittent weekly administration of human parathyroid hormone 1-34 in patients with primary osteoporosis evaluated by histomorphometry and microstructural analysis of iliac trabecular bone before and after 1 year of treatment. J Bone Miner Metab 2004;22:569-76.
61. Cohen A, Dempster DW, Muller R, et al. Assessment of trabecular and cortical architecture and mechanical competence of bone by high-resolution peripheral computed tomography: comparison with transiliac bone biopsy. Osteoporos Int 2010;21:263-73.
62. Jiang Y, Zhao JJ, Mitlak BH, Wang O, Genant HK, Eriksen EF. Recombinant human parathyroid hormone (1-34) [teriparatide] improves both cortical and cancellous bone structure. J Bone Miner Res 2003;18:1932-41.
63. Recker R, Masarachia P, Santora A, et al. Trabecular bone microarchitecture after alendronate treatment of osteoporotic women. Curr Med Res Opin 2005;21:185-94.
64. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. J Bone Miner Res 1999;14:1167-74.
65. Frost HM. Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res 1969;3:211-37.
66. Frost HM. Metabolism of bone. N Engl J Med 1973;289:864-5.

67. Miller PD, Bolognese MA, Lewiecki EM, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. Bone 2008;43:222-9.
68. Watanabe A, Yoneyama S, Nakajima M, et al. Osteosarcoma in Sprague-Dawley rats after long-term treatment with teriparatide (human parathyroid hormone (1-34)). J Toxicol Sci 2012;37:617-29.
69. Andrews EB, Gilsenan AW, Midkiff K, et al. The US postmarketing surveillance study of adult osteosarcoma and teriparatide: study design and findings from the first 7 years. J Bone Miner Res 2012;27:2429-37.
70. Duncan H, Rao SD, Parfitt AM. Complications of Bone Biopsy. In: Jee WS, Parfitt AM, eds. Bone Histomorphometry: THird International Workshop. Paris: Societe Nouvelle de Publications Medicales et Dentaires; 1981:483-6.