The Effect of Etelcalcetide on Bone-tissue Properties and Calcification Propensity in End Stage Kidney Disease

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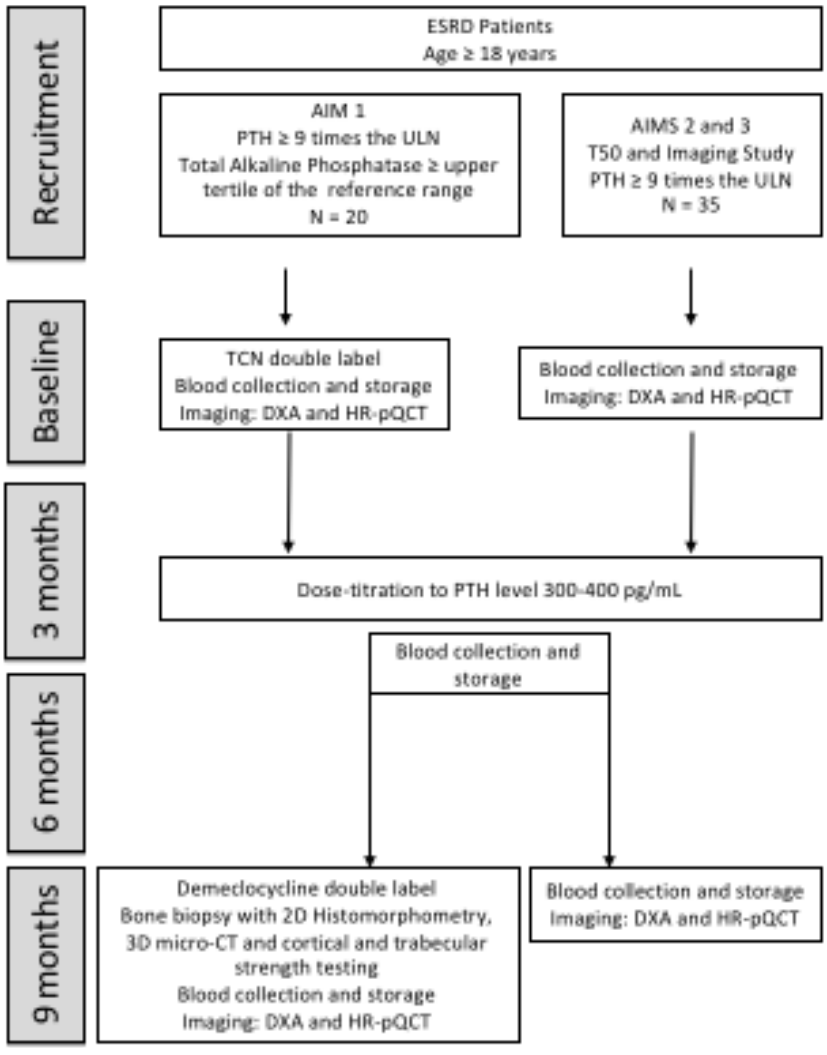
Hypotheses: Our hypothesis is that etelcalcetide treatment improves bone tissue-properties and bone mineral density by reducing remodeling. We also hypothesize that etelcalcetide's ability to lower serum calcium will decrease propensity of serum to calcify as determined by T50.

Background and Study Objectives

Chronic kidney disease – mineral and bone disease (CKD-MBD) is a systemic disorder of bone and mineral metabolism in patients with CKD. As kidney function declines, abnormal vitamin D metabolism and renal phosphate handling drive the development of hyperparathyroidism (HPT), hyperphosphatemia, renal osteodystrophy and soft tissue calcifications. Clinical outcomes associated with CKD-MBD include bone fractures, cardiovascular events and death. Treatment of CKD-MBD is centered around the suppression of parathyroid hormone (PTH) by supplementation with vitamin D (parent and active D) and correction of hyperphosphatemia. In patients with end stage kidney disease (ESKD), target levels of PTH recommended by the Kidney Disease Improving Global Outcomes (KDIGO) guidelines are in the range of 2-9 times the upper limit of normal (ULN) for the PTH assay[1]. However, in patients with long-standing ESKD, abnormalities may develop in the parathyroid gland that both diminish responsiveness to treatment and lead to failure to achieve PTH levels within the KDIGO target range. PTH levels greater than the target range increase the risks of bone loss, fractures, soft tissue calcification and cardiovascular events. In these cases, treatment with a calcimimetic, a pharmacologic agent that enhances sensitivity of the parathyroid glad to serum levels of calcium, can restore PTH levels to goal. Etelcalcetide is a Food and Drug Administration (FDA) approved calcimimetic for the treatment of secondary HPT in adult patients with CKD on hemodialysis[2-4]. The proposed prospective cohort study will investigate the effects of etelcalcetide on the bone and vascular calcification components of CKD-MBD. We will test if treatment with etelcalcetide improves bone properties on the tissue level, increases areal bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA), improves cortical and trabecular BMD and microarchitecture and mechanical competence by high resolution peripheral computed tomography (HR-pQCT), and decreases serum propensity to calcify soft tissues. For all **Aims,** the enrollment scheme and study timeline are described in **Figures 1 and 2**, respectively.

Figure 1. Study Procedures for All Aims



Hypothesis 1. Etelcalcetide improves bone-tissue properties in patients with ESKD and severe hyperparathyroidism.

Aim 1. To test if 6-months of treatment

with etelcalcetide improves bone hardness and mineralization using Ramen/nanoindentation and remodeling by histomorphometry using the quadruple label transiliac crest bone biopsy method in 20 ESKD patients with severe HPT.

Figure 2. Study timeline.

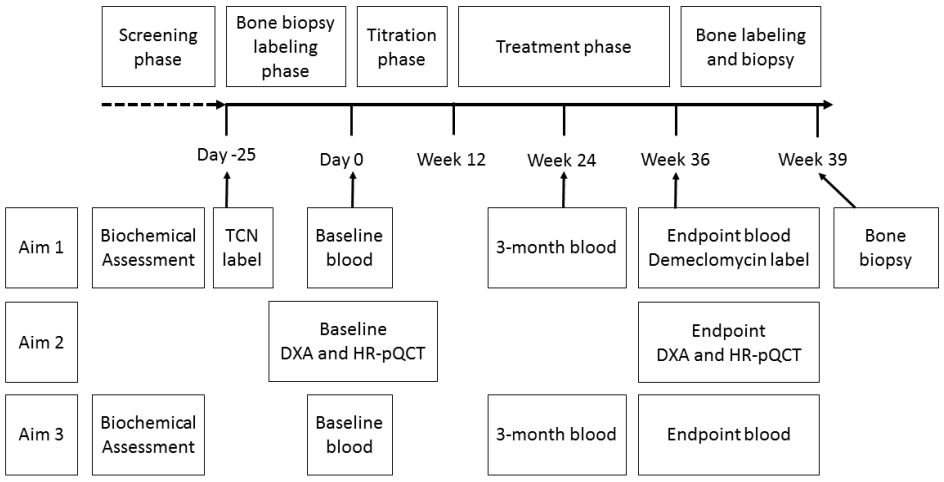


Table 1: Data Collection at each Study Visit			
	Baseline	3 Months	9 Months
Informed consent	x		
Medical History	x		x
Physical Exam	x		x
Kt/V	x	x	x
Biomarkers of bone formation and resorption (BSAP, P1NP, CTX)	x	x	x

PTH, Vitamin D metabolites, Serum calcium and phosphorus, FGF-23, Sclerostin, T50	x	x	x
Bone biopsy Labeling (for participants enrolled in Aim 1)	x		x
Bone biopsy (for participants enrolled in Aim 1)			x
Imaging: DXA and HR-pQCT	x		x
AGE Reader	x		x

Procedures. This is a single-arm prospective cohort study to test the effects of 6-months of treatment with etelcalcetide on bone-tissue properties. We will obtain a single quadruple label bone biopsy to assess longitudinally the effects of 6-months of treatment with etelcalcetide on bone properties at the tissue level. We will enroll 20 adult patients with ESKD on hemodialysis who are scheduled to receive etelcalcetide for the treatment of HPT per standard of care. These 20 patients with ESKD will be treated with etelcalcetide to maintain levels of PTH between 300 and 400 pg/mL, which is at the mid-range of the KDIGO recommended range (2-9 times the ULN for the PTH assay[1]) for 6 months. Patients will be identified for inclusion based on clinical need for the treatment of severe HPT and high turnover bone disease and only patients whose treating physician plans to initiate etelcalcetide will be included. HPT will be defined by the KDIGO guidelines (PTH >9-times the ULN for the PTH assay[1]) and high turnover bone disease will be defined by a total alkaline phosphatase level ≥ upper tertile of the reference range for the assay. At study start, patients will undergo the initial double labeling procedure with tetracycline (TCN; 3-days of TCN followed by 12-days off TCN followed by 3-days of TCN); during the labeling period etelcalcetide will not be administered **(Figure 2**). Patients will then undergo a 3-month etelcalcetide dose-titration phase to ensure that PTH levels are consistently maintained within target range (300-400 pg/mL), followed by a 6-month treatment (maintenance) phase. At the study endpoint (6-months of treatment), the final set of labels for transiliac crest bone biopsy will be given (3-days of demeclocycline followed by 12-days off demeclocycline followed by 3-days of demeclocycline) and bone biopsy will occur 5-days after the last dose of demeclocycline. During the final labeling period, the dose of etelcalcetide will continue to target the goal PTH level and the patient will continue to be managed as clinically indicated. The quadruple label approach permits longitudinal assessment of both dynamic indices of histomorphometry and bone-tissue level properties pre- and post-intervention, with the use of a single biopsy. The first label (TCN) is given prior to the initiation of etelcalcetide and the second label (demeclocycline) is given after 6-months of treatment (maintenance) with etelcalcetide. The fluorescence of tetracycline and demeclocycline differs, permitting differentiation of the labels under fluorescence microscopy and determination of dynamic indices of histomorphometry pre- and post-intervention. To assess bone tissue level effects of treatment, we will use a Raman/Nanoindentation system. This system allows the differentiation of tetracycline (pre-treatment) and demeclocycline (post-treatment) labels and permits precise measurement of localized bone tissue properties (e.g., mineral/matrix, collagen) and indentation properties (e.g., hardness) within these regions. Additionally, we will use 3-dimensional micro-CT (μCT) imaging (resolution 3 μm^3) to obtain more complete structural indices from the bone biopsy specimens at the 6-month time point. Patients will have blood collected and stored at -80ºC, at baseline (pre-treatment) and after 3- and 6-months of treatment (i.e., maintenance phase) (**Table 1**). Blood will be assayed for calcium, phosphorus, PTH, vitamin D metabolites, bone formation (bone specific alkaline phosphatase [BSAP], procollagen type-1 N-terminal propeptide [P1NP]) and resorption (C-Telopeptide [CTX]) markers, FGF-23 and sclerostin.

Bone biopsy with quantitative histomorphometry and nanoindentation/Raman spectroscopy. After bone biopsy, the specimens will be transferred to Dr. Allen's laboratory for tissue analysis (described below).

μCT. 3D trabecular and cortical structure will be assessed on biopsies before embedding using a Skyscan 1172 system (scanning resolution of 6 μm^3). Outcome measurements will include both cortical parameters (porosity [%], mineralization [average value and variation] and thickness) and trabecular parameters (BV/TV, trabecular number, separation, thickness, and connectivity density) using manufacturer software and standard analytical protocols. At study initiation, we will perform short-term precision measurements on selected structural and mechanical properties. QC procedures are enforced by the in-house software system, and include weekly scanning of a QA/μCT phantom. The software system's QA analysis monitors scanner performance and normalizes results for any fluctuations. QA data and specimen results can be exported to SAS or Excel.

Quantitative histomorphometry. Following CT scans, specimens will be embedded in methyl methacrylate using standard protocols. Sections will be cut with a rotary microtome and either left unstained or stained with McNeal tetracrome or tartrate resistant acid phosphatase for analysis. Histomorphometric indices will be defined in accordance with the American Society for Bone and Mineral Research[5]. The following histomorphometric indices will be measured or calculated: osteoid surface, area, and width; osteoclast surface, eroded surface, resorption depth, resorption period; cancellous wall width, mineral apposition rate, bone formation rate, adjusted

apposition rate, mineralization lag time, activation frequency. Note: architectural properties (BV/TV, porosity, etc) will not be collected, as these will be obtained from CT as above.

Bone-tissue properties. We will use an integrated Raman/Nanoindentation system, which permits precise measurement of localized bone tissue properties (e.g., mineral/matrix, collagen) and indentation properties (e.g., hardness) within identical regions. Areas within the TCN and demeclocycline double label will be assessed for nanoindentation properties, and subsequently the same spatial location will be assessed for Raman-based measurements. Nanoindentation will occur using a Berkovich diamond indenter loading to a peak load of 10mN, a strain rate of 0.05 seconds and a holding period of 10 seconds. Oliver and Pharr methods will be used to determine the stiffness and hardness of the unloading curves. A total of 12-15 indentations will be performed within a given region. Following indentation, the same spatial regions will be assessed with Raman spectroscopy to determine the phosphate and matrix peaks after background and baseline shift correction. The ratio of phosphate:mineral will be calculated, along with other parameters (crystallinity and collagen crosslinking).

Hypothesis 2. Etelcalcetide will improve bone density, microarchitecture, and biomechanical competence in patients with ESKD.

Aim 2. To test if 6-months of treatment with etelcalcetide improves femoral neck areal BMD measured by DXA and failure load at the ultradistal radius measured by application of finite element analysis to HR-pQCT datasets. We will also quantify the effect of 6-months treatment with etelcalcetide on changes in areal BMD at the spine, total hip, and one-third and ultradistal radius measured by DXA, and cortical and trabecular volumetric BMD and microarchitecture, cortical porosity and tissue mineral density, and biomechanical competence at the ultradistal radius and tibia measured by HR-pQCT with application of finite element analysis.

Procedures. We will enroll 35 patients (20 from Aim 1 and 15 *de novo*) into this prospective single-arm cohort study to test the hypothesis that 6 months of treatment (maintenance) with etelcalcetide will improve bone density, microarchitecture and mechanical competence measured by DXA and HR-pQCT with application of finite element analysis (FEA). Similar to **Aim 1**, eligibility for enrollment into **Aim 2** will be based on a PTH level >9-times the ULN for the PTH assay (**Figure 1**), as well as their treating physician's plan to initiate etelcalcetide per standard of care. However, the presence of high turnover bone disease based on total alkaline phosphatase will not be a criterion for enrollment into **Aim 2**. Furthermore, all patients enrolled in **Aim 1** will be automatically enrolled into **Aim 2** unless they choose to opt-out of **Aim 2**. As per **Aim 1** (**Figure 2**), after enrollment, patients will undergo a 3-month dose titration phase to ensure that PTH levels are stability maintained within the target rage (300-400 pg/mL), followed by a 6-month treatment (maintenance) phase. All patients will have imaging (DXA and HR-pQCT) performed at baseline (prior to drug) and after 6-months of maintenance treatment.

Imaging Methods for Measurement of Bone Mass, Microarchitecture, and Mechanical Competence

- **DXA.** areal BMD of spine (L1-4, AP), right proximal femur, forearm and whole body using Hologic QDR 4500 (Hologic, Inc., Waltham, MA) in the array (fan beam) mode. Phantoms are scanned daily for QA. Short term, *in vivo* precision is 0.68%, 1.36%, and 0.70% for spine, FN, and radius respectively.
- **HR-pQCT of the ultradistal radius and tibia**. XtremeCT2 (Scanco Medical AG, Brüttisellen, Switzerland, voxel size 60μm). Methods and QC are described in our publications[6, 7]. Precision: cortical and volumetric trabecular BMD=0.9%±7.1% and 2.3±3.5%, respectively. HR-pQCT of the forearm will be performed at the non-dominant forearm. If dialysis access is located at the non-dominant forearm, we will use the dominant forearm for HR-pQCT imaging.

Mechanical Analysis of Skeletal Imaging. Sanchita Agarwal MS will perform analyses of HR-pQCT datasets. **HR-pQCT Finite Element Analysis (FEA)**. Ms. Agarwal will estimate mechanical competence (strength) from HR-pQCT images. Each image is converted to a μFE model by directly converting bone voxels to 8-node elastic brick elements. A uniaxial displacement equaling 1% of the bone segment height is applied perpendicularly to the distal surface of the radius or tibia while the proximal surface is imposed with zero displacement along the same direction. The total reaction force is calculated from the linear μFE analysis, and the axial stiffness is calculated as the reaction force divided by the imposed displacement.

HR-pQCT Cortical Porosity. Ms. Agarwal will conduct analyses of cortical porosity using methods that have been described and validated in recent publications[8].

Hypothesis 3. Etelcalcetide decreases serum calcification propensity in patients with ESKD.

Aim 3. To test if 6-months of treatment with etelcalcetide decreases serum propensity to calcify soft tissues. In a single-arm study of 35 patients with ESKD treated with 6-months of etelcalcetide, we will measure T50, calciotropic hormones, serum calcium and phosphorus, bone turnover markers, FGF-23 and Sclerostin pre-treatment and 3- and 6-months after treatment.

Procedures. We will enroll 35 patients (20 from Aim 1 and 15 *de novo*) into this prospective single-arm cohort study to test the hypothesis that 6 months of treatment (maintenance) with etelcalcetide will decrease the propensity to calcify soft tissues as measured by T50. Similar to **Aim 1**, eligibility for enrollment into **Aim 3** will be based on a PTH level >9-times the ULN for the PTH assay (**Figure 1**), as well as their treating physician's plan to initiate etelcalcetide per standard of care. However, the presence of high turnover bone disease based on total alkaline phosphatase will not be a criterion for enrollment into **Aim 3**. Furthermore, all patients enrolled in **Aim 1** will be automatically enrolled into **Aim 3** unless they choose to opt-out of **Aim 3**. Thus, we will use the biochemical data from the 20 enrollees participating in **Aim 1** and enroll an additional 15 patients to be treated with etelcalcetide. As per **Aim 1** (**Figure 2**), after enrollment, patients will undergo a 3-month dose titration phase to ensure that PTH levels are stability maintained within the target range (300-400 pg/mL), followed by a 6-month treatment (maintenance) phase. Patients will have blood collected and stored at -80ºC, at baseline (pre-treatment) and after 3- and 6-months of treatment (i.e., maintenance phase). Blood will be assayed for T50, calcium, phosphorus, PTH, vitamin D metabolites, bone formation (BSAP, P1NP) and resorption (CTX) markers, FGF-23 and sclerostin. We will assess the effect of treatment on changes in levels of T50, a clinically relevant biomarker of calcification propensity[9, 10]. We will also determine relationships between changes in T50 and changes in phosphorus, calcium, bone turnover markers and calciotropic hormones.

Exploratory. None

Primary and Secondary Endpoints for Each Aim

Aim 1

1. Primary Endpoints: Hardness
2. Secondary Endpoints: Bone formation rate, mineralization density

Aim 2

1. Primary Endpoints: Changes in: (a) femoral neck areal BMD by DXA and (b) radius failure load by HR-pQCT
2. Secondary Endpoints: Changes in: (a) spine, total hip, and one-third and ultradistal radius BMD by DXA and (b) radius and tibia cortical density, thickness, porosity and tissue mineral density, and trabecular density, thickness, number and spacing

Aim 3

1. Primary Endpoint: Change in T50 (percent and absolute)
2. Secondary Endpoints: Changes in PTH, calciotropic hormones, FGF-23, Sclerostin, calcium and phosphorus

Statistical methods (for analysis of primary and secondary endpoints or primary/interim analysis)

For All Aims, distributions will be examined and Anderson-Darling tests of normality and Q-Q plots will be used to guide the application of transformations to normalize the values prior to inferential testing. (Data analyzed from transformations will be reported in raw units). Inferential tests will be adjusted for multiple comparisons within a measurement domain (BMD within DXA, cortical, trabecular and FEA within HRpQCT, and each of the four compartments within histomorphometry) using a resampling, step-down procedure to control the family-wise error rate. We favor this over a false-discovery rate approach given the limited number of hypotheses within each measurement domain and that the measurement domains are traditionally seen as free to vary independently of each other[11, 12].

For Aim 1. A two-tailed dependent T-test of the change in hardness measured by Ramen nano-indentation and mineralization measured by histomorphometry obtained primary dynamic and secondary structural bone quality

outcome values from the pre-treatment baseline to the 6-month endpoint will be reported with an alpha-level of 5% as the criterion for statistical significance. Outcome data missing, for whatever reason, will not be imputed.

For Aim 2. A two-tailed dependent T-test of the change in femoral neck BMD by DXA and failure load measured at the distal radius by HR-pQCT from pre-treatment to the 6-month endpoint will be reported with an alpha-level of 5% as the criterion for statistical significance. Secondary endpoints include treatment differences in change in cortical density, thickness and porosity, trabecular density and thickness and spacing, and areal BMD at the forearm, spine, and total hip. Other predictors to be tested in multiple regression models of change from baseline include demographic characteristics collected in **Aim 1** and PTH, calciotropic hormones, bone formation and resorption markers, sclerostin, and FGF-23 collected in **Aim 3**. A final multiple regression model will be based on predictors that are significant in multiple regression models including baseline bone measure.

For Aim 3. A two-tailed dependent T-test of the change in primary calcification propensity (T50) and secondary biochemical indices (calciotropic hormones, FGF-23, sclerostin, calcium and phosphorus) from pre-treatment baseline to the 6-month endpoint will be reported with an alpha-level of 5% as the criterion for statistical significance. Secondary exploratory analyses will assess the within-subject temporal course of change in the biochemical outcome with linear mixed models for repeated measures with time as a fixed effect (0, 3 and 6 months); the baseline value of the dependent variable entered as a continuous covariate; and a covariance structure determined by empirical testing performed prior to inferential testing. We will also assess whether the **Aim 1** participants differ from the patients recruited *de novo* for **Aim 3** with an independent T-test both of the two groups' baseline values and of the two groups' change in values from baseline to the 6-month endpoint. We will explore the covariances between changes in T50 and other biochemical markers of bone turnover and calciotropic hormones with simple and multiple regression with synchronized and lagged observations in time to determine whether leading, cotemporaneous or following patterns exist among these measures.

Subject Recruitment and Selection

Study site and number of patients eligible for enrollment. ESKD patients receiving hemodialysis at the Rogosin Institute, meeting enrollment criteria, and whose treating physicians intend to initiate treatment with etelcalcetide will be asked to participate in this study. Rogosin is the largest provider of hemodialysis in New York City and provides hemodialysis services to over 1300 patients. Approximately 25% of patients at Rogosin have PTH levels > 9-times the ULN for the PTH assay. Therefore, there are adequate numbers of patients at Rogosin who may be able and willing to participate in this prospective cohort study.

Enrollment Criteria

Inclusion criteria

For All Aims:

1. Patient has provided informed consent.
2. Patient is 18 years of age or older.
3. Patient must be receiving maintenance hemodialysis for at least 3 months, with adequate hemodialysis with a delivered Kt/V 1.2 or urea reduction ratio (URR) 65% within 4 weeks prior to screening laboratory assessments.
4. Dialysate calcium concentration must be stable for at least 4 weeks prior to screening laboratory assessments.
5. Patient must have severe HPT as defined by two laboratory screening pre-dialysis serum PTH values >9-times ULN for the PTH assay, measured on two consecutive monthly lab checks prior to entering the study.
6. The patient has an uncontrolled PTH defined by KDIGO as a PTH greater than 9 times the upper limit of normal of the assay (720 pg/mL for Rogosin):

   AND one of the following:
   - The patient has never been on cinacalcet OR,
   - The patient received daily cinacalcet for less than 3 months and has been off cinacalcet for at least 3 months prior to enrollment OR ,

- The patient received daily cinacalcet for more than 3 months and has been off cinacalcet for at least 6 months prior to enrollment OR,
- The patient received a modified dose of three times weekly cinacalcet and has been off cinacalcet for at least one month prior to enrollment.

7. Scheduled to receive etelcalcetide for the treatment of HPT per standard of care.
8. If receiving vitamin D sterols, patient must have had no more than a maximum dose change of 50% within the 4 weeks prior to screening laboratory assessments, remain stable through randomization, and be expected to maintain stable doses for the duration of the study, except for adjustments allowed per protocol*.
9. Patient must have one screening pre-dialysis serum Ca laboratory value at least at the lower limit of normal for the assay measured within 4 weeks prior to entering the study.
10. A patient receiving calcium supplements must have had no more than a maximum dose change of 50% within 2 weeks prior to screening laboratory assessments and remain stable throughout the study, except for adjustments allowed per protocol*.
11. A patient receiving phosphate binders must have had no more than a maximum dose change of 50% within the 2 weeks prior to screening laboratory assessments, remain stable through, and be expected to maintain stable dose for the duration of the study, except for adjustments allowed per protocol*.
12. The treating physician considers the etelcalcetide dose and timing points described in this protocol as acceptable/optimal for their patient.
13. Female patients must be willing to use highly effective contraception during the study and for 3 months after the last dose of etelcalcetide (unless postmenopausal or surgically sterilized).

For Aim 1:

1. Total alkaline phosphatase ≥ the upper tertile of the reference range for the assay

Exclusion criteria

For All Aims:

1. Currently receiving treatment in an investigational device or drug study, or less than 30 days since ending treatment on an investigational device or drug study(s).
2. Currently receiving investigational procedures while participating in this study.
3. Patient with controlled PTH as defined by KDIGO as a PTH of 2 to 9 times the upper limit of normal of the assay.
4. Patients has received a bisphosphonate, denosumab or teriparatide during the 12 months prior to screening.
5. Anticipated or scheduled parathyroidectomy during the study period.
6. Patient has received a parathyroidectomy within 6 months prior to dosing.
7. Scheduled kidney transplant during the study period or anticipated living donor evaluation within three months of recruitment
8. Patient has an unstable medical condition based on medical history, physical examination, and routine laboratory tests, or is otherwise unstable in the judgment of the Investigator.
9. Bilateral lower extremity amputations or non-ambulatory
10. Metabolic bone diseases not related to the kidney (i.e., Pagets, Osteogenesis Imprefecta)
11. Untreated hyperthyroidism or hypoparathyroidism
12. Malignancy within the last 5 years (except non-melanoma skin cancers or cervical carcinoma in situ).
13. Patient is pregnant or nursing.
14. Patient likely to not be available to complete all protocol-required study visits or procedures, and/or to comply with all required study procedures to the best of the patient and Investigator's knowledge.
15. Weight >300 pounds

For Aim 1:

1. Allergy to tetracycline or demeclocycline.

Early Study Cessation Clause

For patients who may get a kidney transplant during the study: If patients have been on etelcalcetide for at least 3 months, end of study procedures (bone biopsy, bone imaging, and labs) will be performed and patients will be included in the final analysis as long as patients come in within 4 weeks after transplant.

*** Administration and Dosing of Etelcalcetide and Management of Hypocalcemia and Over-suppression of PTH**

The protocol will allow for changes in the dose of etelcalcetide, vitamin D sterols, calcium supplements and phosphate binders based on the levels of serum PTH, calcium and phosphorus that are obtained on a monthly basis as part of the standard of care monthly lab set for patients on hemodialysis.

1) Administration: by intravenous bolus injection into the venous line of the dialysis circuit at the end of hemodialysis treatment during rinse back or intravenously after rinse back.

2) Initiation and dose adjustment of etelcalcetide
   a) Starting dose of etelcalcetide: 5.0 mg
   b) Maximum dose of etelcalcetide: 15.0 mg
   c) Dose adjustments: increase in 2.5-5.0 mg increments at weeks 4, 8, 12 during the titration phase and at the time of the monthly lab sets during the treatment phase based on PTH and calcium.

3) Missed doses
   a) If a regularly scheduled hemodialysis treatment is missed, do not administer any missed doses.
      i) Resume etelcalcetide at the end of the next hemodialysis treatment at the prescribed dose.
      ii) If doses of etelcalcetide are missed for more than 2 weeks, re-initiate etelcalcetide at the recommended starting dose of 5 mg (or 2.5 mg if that was the patient's last dose).

4) Management of hypocalcemia
   a) Monitor corrected serum calcium one-week after starting or changing a dose of etelcalcetide
   b) Drop in corrected serum calcium WNL of the reference range
      i) no dose change
   c) Drop in serum calcium < LLN of the reference range but ≥ 7.5 mg/dL without symptoms of hypocalcemia
      i) increase calcium in dialysate to the next highest level and/or start calcium oral supplement and monitor as per HD unit safety protocol
         (1) If calcium has normalized and PTH remains within the target range: no dose change
         (2) If calcium has normalized but PTH has risen above the target range: increase dose by 2.5 mg and monitor PTH and calcium per HD unit safety policy
            (a) If calcium and PTH are at goal: no dose change
            (b) If calcium < LLN but ≥ 7.5 mg/d without symptoms: increase calcium in dialysate and/or start calcium oral supplement and monitor as per HD unit safety protocol
               (i) If calcium remains < LLN but ≥ 7.5 mg/d, changes in levels of active vitamin D sterols can be implemented
                  1. Decreasing the dose of etelcalcetide can be implemented after the above steps have been undertaken. The dose should be lowered in 2.5 mg increments and monitoring of PTH and calcium should be obtained within a week of any changes.
   d) Drop in serum calcium < 7.5 mg/dL or below the reference range with symptoms of hypocalcemia
      i) Hold drug and monitor as per HD unit safety protocol
         (1) If repeat calcium level WNL: restart dose at 2.5-5.0 mg lower than the previous dose and monitor as per HD unit safety protocol
            (a) If repeat calcium level remains WNL and PTH at goal: no dose change
            (b) If repeat calcium level remains WNL and PTH above goal: increase dose by 2.5 mg and monitor per HD unit safety protocol
               (i) If calcium level < LLN but ≥ 7.5 mg/d without symptoms: follow algorithm 4c
               (ii) If calcium level < 7.5 mg/dL or below the reference range with symptoms of hypocalcemia: proceed as per 4di

5) Management of excessive suppression of PTH below the KDIGO target (< 2x LLN)

i) Measure PTH after 4 weeks from initiation or dose adjustment
ii) Without hypocalcemia: decrease dose by 2.5-5.0 mg and monitor as per HD unit safety protocol
iii) With hypocalcemia: as per (4) above

Sample Size for Aim 1. N = 20
Sample Size Justification for Aim 1. The proposed sample size of 20 participants provides 80% power and 5% alpha for a two-tailed dependent T-test of the within-subject difference in the pre- and post-treatment hardness measured by Ramen/nanoindentation in the formation regions of 0.91-SDs. While we expect moderate variability in hardness between subjects, the within subject change due to treatment sought here is expected to be relatively similar across subjects given the established efficacy of etelcalcetide on other outcomes[13]. Behets et al[14] reports patients with end stage renal disease show a baseline bone formation rate of 799 ± 334 (mean ± SD), a O.Pm/B.Pm(%) of 18.9 ± 9.4 and a E.Pm/B.Pm of 13.1 ± 4.3 which assures a measurable region for the Ramen/nanoindentation assessment of hardness.

Sample Size for Aim 2. N = 35 (20 from Aim 1 plus 15 additional patients)
Sample Size Justification for Aim 2. The proposed sample size of 35 participants provides >80% power and 5% alpha to detect a change in femoral neck BMD of 0.5% f and a 0.5 standard deviation change in radius failure load by HR-pQCT from pre-treatment to the 6-month endpoint. For femoral neck BMD by measured by DXA, Lien et al [15] reported that in 8 patients with CKD treated with cinacalcet for 6 months, areal BMD at the femoral neck increased 2.2 ± 1.1% ($p < 0.05$). For failure load outcomes, in a longitudinal study of 29 patients with ESKD who underwent kidney transplantation, we reported that failure load decreased by 3.5 ± 7.0% ($p=0.0081$) over 12-months[16]; our larger sample size will permit detection of at least a 3.4% change in radius failure load due to suppression of PTH production by etelcalcetide.

Sample Size for Aim 3: N = 35 (20 from Aim 1 plus 15 additional patients)
Sample Size Justification for Aim 3: The proposed sample size of 35 participants provides >80% power and 5% alpha two-tailed dependent T-test of the change in T50 from baseline to the 6-month endpoint. Based on Smith et al[10], each standard deviation (SD) decrease in T50 was associated with a 39% increase in odds of all-cause mortality. Assuming that the variability in T50 in patients on hemodialysis patients enrolled into this prospective cohort study are similar to those reported in Smith et al[10], approximately 20 patients would be required to detect a 1-SD decrease in T50. Our sample size of 35 patients will permit the detection of a 0.7-SDs decline in T50. We assume that a reduction of lesser magnitude is of unclear clinical significance

Protection of Human Subjects
This study qualifies as clinical research and will be conducted in compliance with GCP and all regulatory requirements for the protection of human subjects.

Informed Consent
Informed consent will be obtained from all participants. The purpose of the study will be explained to patients prior to recruitment as part of the informed consent procedures, which is defined as "to evaluate the effects of etelcalcetide on bone quality and the tendency of your soft tissue to calcify". The study methods, namely the assessment of physical exam and medical history; the evaluation by imaging modalities; the collection of blood; and bone biopsy will be described. The fact that the informed consent and the study protocol have been approved by the IRB will be emphasized. The potential risks and direct benefits to the patient will be explained. The potential benefit from this study to future patients will be explained. The fact that the patient has the option not to participate and will receive the same standard medical care regardless of participation will be emphasized. The rights of the patient, including the voluntary nature of participation and the right to withdraw the consent at any time, will be explained. We will also explain that this research does not affect the clinical management of the patient as determined by their nephrologist. In addition, patients will be informed that by enrolling they will have more intensive monitoring of bone health than they would if they do not participate. Thus, participating in this study should not put the subject at any risk for altering management deemed appropriate by the patient's nephrologist. The methods in place for maintaining patient confidentiality will be explained. This will include registering all data under a subject specific alpha-numeric system, storing all records in password protected and encrypted files. A copy of the signed informed consent will be placed in the patient's permanent medical record, a second copy of all consents will accompany the data to be kept in the PI's office, and a third copy will be provided to the participant.

Potential Risks
All key personnel involved in the design or conduct of research involving human subjects will have received the required education on the protection of human research participants prior to funding of this project.

The potential risks are related to hypocalcemia, loss of confidentiality, radiation exposure from DXA for measurement of bone density and HR-pQCT for measurement of volumetric density and microarchitectural parameters and the bone biopsy procedure. All blood will be obtained from the dialysis line to avoid venipuncture.

- **Hypocalcemia.** Etelcalcetide has been associated with a decrease in serum calcium. We will monitor for and minimize this risk as described in the main protocol.
- **Loss of confidentiality.** due to breaches in data security or deductive disclosure. To ensure that participants' confidentiality is not compromised, we will minimize these risks through the procedures discussed below.
- **Radiation.** The total expected radiation dose for the entire study is approximately 8 mrem (80 μSv) for 2-sets of DXA and HR-pQCT scans obtained at the baseline and 12-month visits. This compares to 2400 μSv natural background in a year, 8000 μSv for a standard chest CT, 450 μSv for a mammogram, and 60 μSv for a round-trip transcontinental plane flight. The total radiation exposure for the study will be below the maximal acceptable dose.
  a) **CUMC DXA (2 sets of scans).** Radiation exposure for DXA (spine, one hip, forearm) using the Hologic QDR4500 (fast scan) is 10.85 μSv (21.68 μSv for 2 sets of scans).
  b) **HR-pQCT (one forearm and one leg; 3 sites each).** 30 μSv (60 μSv for 2 sets of scans)
- **Transiliac crest bone biopsy.** The risks of the bone biopsy include those related to tetracycline and demeclocycline administered in advance of the biopsy to label sites of bone formation, the risks of conscious sedation, and the risks of the biopsy itself. Tetracycline-type agents may cause stomach upset and skin rashes when subjects taking them are exposed to sunlight. Subjects will be counseled to discontinue tetracycline/demeclocycline if gastrointestinal symptoms develop and to avoid sunlight exposure during the 6 days they are taking the medication. Conscious sedation will be offered to subjects in order to minimize anxiety, provide pain relief, and diminish recall. Patients receiving conscious sedation may have brief, transient loss of protective reflexes. Either IV or PO sedation will be provided with a combination of a benzodiazepine (e.g, midazolam, valium) and a narcotic-based pain medication (e.g., fentanyl, oxycodone). Risks will be minimized by complying with all procedures as required by CUMC's Policies and Procedures Manual. Specifically, all nurses and physicians involved in performing bone biopsies will have certification in Advanced Cardiac Life Support, the procedure will be conducted in the Biopsy Suite in the Division of

Nephrology at CUMC, and vital signs (including O_2 saturation) will be monitored at regular intervals during and after the procedure. An M.D. or Nurse-anesthetist will be present to administer the conscious sedation. Complications from the biopsy are very unusual. Occasionally there will be a bruise at the site or discomfort at the biopsy site that persists for a few days.

Protection against risk
The risks of DXA, HR-pQCT, bone biopsy and loss of confidentiality are described above. Provision for medical treatment, should it be required, will be included in the written informed consent; each subject will receive a copy. Confidentiality of patient data will be ensured. No patient will be identifiable in any publications resulting from this study. Patients are identified by code rather than name in the database. Access to the computer data bank is limited by password protection.

- **Hypocalcemia.** We have implemented a protocol to monitor and manage hypocalcemia.
- **Radiation.** DXA, and HR-pQCT have the risk of radiation exposure. The amount of radiation that participants will receive as a result of participation in the study will be discussed with each participant in detail as part of informed consent procedures. Females will undergo a urine pregnancy test prior to the DXA and HR-pQCT. Pregnant females will be excluded from the study at any time point in order to protect the unborn fetus.
- **Bone biopsy.** The risks of bone biopsy will be included in informed consent procedures. Furthermore, our use of a quadruple label method negates the need for 2 bone biopsy procedures.
- **Confidentiality.** Only investigators and the study research staff will have access to the data. All data are recorded with linkages to subject identities to be able to track changes over their longitudinal participation in the study. A data collection/tracking system developed for use in previous studies is used in this research, and will ensure confidentiality for all participants. All collected data will be identified by study identification numbers but not names. Only the study investigators will have access to a master list with the study identification numbers and names. The master list will be kept on a secure server on a research shared access network. All data containing PHI will be stored on password-protected encrypted CUMC computer or the secure research servers. Computers are password protected, encrypted and regularly backed-up to prevent data loss. Paper documents, such as consent forms, will be stored in locked cabinets in areas of restricted access. Only the research staff will have access to this information, unless written permission is received from the participant. The data collected as part of this study will be retained indefinitely. All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy, and the investigators and other site personnel will not use such data and records for any purpose other than conducting the study.

Safety Reporting
The Principal Investigator will be responsible for patient safety. As this is not a clinical trial, there is minimal potential for injury.

Adverse events will be monitored throughout the study and reported to Amgen as follows:
- Suspected Unexpected Serious Adverse Reaction (SUSARs) will be submitted at the time of regulatory submission
- Pregnancy/Lactation Reports will be submitted within 10 calendar days of Sponsor awareness
- Applicable Annual Safety Reports will be submitted annually
- Other Aggregate Analyses (any report containing safety data generated during the course of the study) will be submitted at the time of ISS Sponsor submission to the IRB (or other body governing research conduct)
- The Final (End of Study) Report will be submitted to Amgen at the time of ISS Sponsor submission to the IRB (or other body governing research conduct), but not later than 1 calendar year of study completion

Any unanticipated problem involving risks will be reported to the CUMC-IRB in accordance with their Reporting Policy effective January 24, 2008.

Additionally, throughout the study, the Principal Investigator will review safety labs that are ordered per standard of care, and may provide recommendations to the treating physician based upon the drug dosing and management guidelines included in this protocol.

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