The Effects of 12-months of Denosumab on Bone Density, Quality and Strength in Prevalent Kidney Transplant Recipients

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Key Personnel
Donald J. McMahon: Statistician

A. HYPOTHESES AND AIMS

In 2014, >17,000 kidney transplantations were performed, >200,000 persons were living with a functioning transplant, and >90% were expected to live beyond the third post-transplant year. Bone fractures are 3-times more common in kidney transplant recipients than in the general population[1], one-quarter of recipients were reported to sustain a fracture within the first 5-years of transplantation[2], and after hip fracture mortality risk increased by up to 60%[3]. There are no proven anti-fracture strategies for kidney transplant recipients. Studies demonstrating anti-fracture efficacy of therapies proven to prevent fracture in recipients of other solid organs[4] and in patients with age-related and glucocorticoid-induced osteoporosis have not been conducted in kidney transplant recipients. Thus, there is an unmet clinical need to develop and study strategies that prevent fractures in kidney transplant recipients to improve long-term skeletal outcomes and survival.

Prevention of fractures after kidney transplantation with bisphosphonates is controversial due to their potential for nephrotoxicity. Furthermore, bisphosphonates are cleared by the kidney and there is concern that over-accumulation in bone may occur in patients with < 30% of kidney function, resulting in over-suppression of bone turnover. Denosumab, a monoclonal antibody against RANKL, inhibits osteoclast function and is not cleared by the kidney. Denosumab prevents fractures in patients with age-related osteoporosis and safely prevents fractures in immunosuppressed patients with glucocorticoid-induced osteoporosis. Recently, a non-blinded randomized trial in 90 patients during the first year of kidney transplantation that compared denosumab to routine therapy, demonstrated that denosumab suppressed biomarkers of bone turnover, increased bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) at the spine and hip by 5.1% and 1.9% respectively[5], and in a subset of 10 patients who underwent imaging by high resolution peripheral quantitative computed tomography (HR-pQCT) and saw an increase in estimated bone strength by 3.9% at the tibia using finite element analysis[6]. Adverse events in denosumab-treated patients included higher incidence of urinary tract infections, diarrhea, and transient asymptomatic hypocalcemia[5,7]. This study demonstrated that denosumab safely increased BMD at the spine and hip in de-novo kidney transplant recipients. However, long-term kidney recipients who comprise the vast majority of patients living with a transplanted kidney, and who are also at increased risk of fracture, were not included.

To generate additional data that will support the need for a clinical trial to assess efficacy of denosumab for prevention of fracture in kidney transplant recipients, we now propose to conduct a multi-center double-blinded placebo-controlled randomized trial of 60 patients (40 treatment, 20 placebo) treated with twice yearly denosumab for 1-year to test the effect of denosumab on BMD in the long-term kidney transplant recipient population. We hypothesize that denosumab versus placebo will safely improve bone density by DXA. We also hypothesize that denosumab versus placebo will improve bone quality and strength as assessed by HR-pQCT in the subset of patients recruited at Columbia University Irving Medical Center (CUIMC).

Aim 1. To determine if 1-year of treatment with denosumab versus placebo increases BMD by DXA. Sixty (40 Denosumab / 20 Placebo) patients will undergo testing of BMD at the spine, hip and forearm by DXA at enrollment, 6-months and end of treatment.

Aim 2. To determine if bone mechanical competence measured by HR-pQCT with application of micro-finite element analysis is improved by 1-year of denosumab treatment compared to controls. A subset of 20 (10/group) participants will undergo HR-pQCT imaging of the radius and tibia at baseline, 6-months and end of treatment. Changes in bone mechanical competence (hardness) and cortical and trabecular microarchitecture will be quantified and compared between the two treatment groups.

B. INVESTIGATORS AND STUDY SITES

B.1. Principal Investigators

B.1.a. Lead Study Site (CUIMC Site)

Columbia University Irving Medical Center, NY, NY

Thomas L. Nickolas MD MS is a federally funded investigator and internationally renowned expert in the biology, physiology and epidemiology of renal osteodystrophy and mineral disorders that occur in CKD.

B.1.b. Participating Sites (Non-CUIMC Sites)

Northwestern University Medical Center, Chicago, IL

Tamara Isakova MD MMSc is a federally-funded investigator and internationally renowned expert in the biology, physiology and epidemiology of mineral metabolism in CKD.

NorthShore University HealthSystem, Evanston, IL; University of Chicago, Chicago, IL

Stuart M. Sprague DO is the Chief of Nephrology at NorthShore University HealthSystem and Clinical Professor of Medicine at University of Chicago Pritzker School of Medicine, he is an internationally recognized expert in mineral and bone disorders in CKD. He is affiliated with University of Chicago, whose patients will be referred to NorthShore University for study procedures.

B.2. Co-Investigators

Donald J. McMahon MS is a senior biostatistician. He has worked for over 20 years in metabolic bone disease and has overseen the design, implementation, data collection, and data analysis of multiple NIH funded studies on metabolic bone diseases.

B.3. Population and Recruitment Locations: We will enroll prevalent kidney transplant recipients at four clinical sites. The centers and their characteristics and numbers of prevalent transplant recipients meeting our study's inclusion criteria are in **Table 1**:

Table 1: Proposed Recruitment Centers	**Prevalent Transplant Patients**	**New Transplants per Year**	**DXA Capable**	**HRpQCT Capable**
Columbia University Irving Medical Center, NY, NY	3000	200	+	+
Northwestern University Medical Center, Chicago, IL	1000	250	+	
NorthShore University Health System, Evanston, IL	200	15	+	
University of Chicago, Chicago, IL*	1150	80	+	

***Potential patients will be referred for recruitment to NorthShore University**

B.4. Investigator and Research Team Meetings

The PIs and clinical research coordinators at all locations will have teleconferences by video and audio link on a weekly basis after the first recruited patient for the first six months and on a bimonthly basis afterwards. Ad hoc meetings will occur on an as needed basis to discuss adverse events, protocol deviations, protocol changes and/or other issues that affect study execution and management.

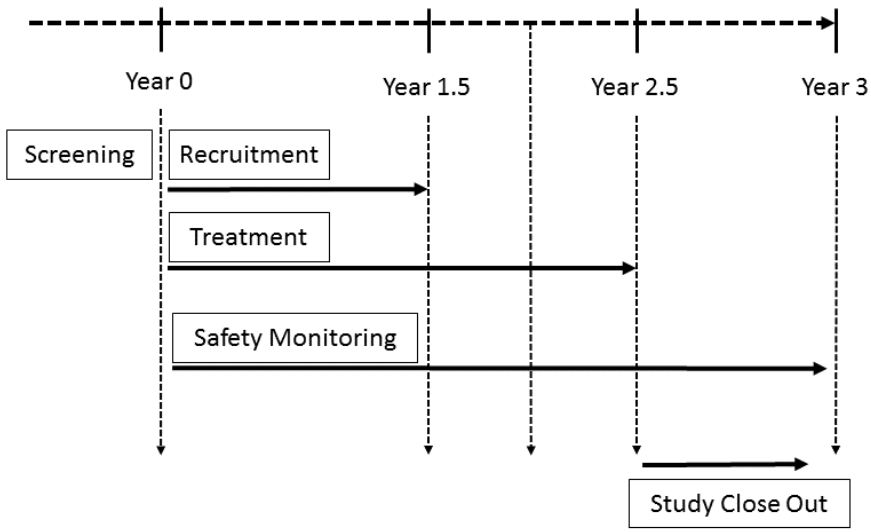
C. STUDY TREATMENT

C.1. Study Drug and placebo: Denosumab is an anti-resorptive agent; it is monoclonal antibody against RANKL (receptor activator of NkB ligand) and inhibits osteoclast development and function. Denosumab 60 mg is given as a SQ injection every 6-months. This RCT will use a placebo comparator group because there is no approved treatment for low bone mass and increased fracture risk in kidney transplant recipients. Active drug and placebo will be provided by the manufacturer and both will be given as a subcutaneous injection every 6 months for 12-months.

D. STUDY PROCEDURES

D.1.a. Study Timeline: This is a 3-year study (**Figure 1**). Sixty men and women (40 Denosumab / 20 Placebo) ≥ 18-years, who are ≥ 12 months after kidney transplantation, with GFR ≥ 30 mL/minute/1.73 m^2 (MDRD or CKD-EPI per local lab reporting), and stable allograft function will be enrolled over the first 1.5-years of the study, treated with 12-months of denosumab or placebo, and followed until end-of-study for post-treatment complications.

Figure 1: Study Timeline



D.1.b. Participant Remuneration: All patients will receive a total of $400.00 over the course of the study. This will be distributed to them at each visit according to the following payment schedule:

- Screening Visit: $25
- Baseline Visit: $75
- 2-weeks post-baseline Visit: $25
- 1-week pre 6-month Visit: $25
- 6-months Visit: $75
- 2-weeks post-6 month Visit: $25
- 12-months Visit: $150

D.2. Recruitment, Informed Consent, Screening & Evaluation, Enrollment & Randomization, Study Treatment, and Post-Treatment Follow-up and Bone Care

D.2.1. Recruitment: Potential participants will be identified based on inclusion / exclusion criteria itemized below. After permission to approach the patient is provided by the patient's nephrologist, the study will be explained to them. If they agree to further screening to determine if they adhere to the full eligibility criteria, informed consent (ICF) will be obtained. After consent is obtained, screening to rule out the following exclusion criteria will occur: (1) assessment of bone mineral density (BMD) to rule out a T-Score ≥ -1.0 at the spine; (2) thoracic and lumbar spine X-rays to assess for occult vertebral fractures; and (3) assessment for serum calcium ≤ 9.0 mg/dL and 25OHD ≤ 30 ng/mL.

D.2.1.a. Inclusion and Exclusion Criteria We will include 60 men and women who are ≥12 months after kidney transplantation with stable allograft function. We will exclude patients expected to need dialysis and/or die within two-years of enrollment, and medical conditions that may alter fracture risk independent of transplantation effects. Given the risk of hypocalcemia associated with denosumab, we will ensure that all patients have pre-treatment levels of serum calcium and 25OHD of ≥ 9.0 mg/dL and ≥ 30 ng/mL respectively. Due to the known risk of osteonecrosis of the jaw, we will ensure that all participants are receiving dental care, are in good dental health, and will be excluded if they will be having a dental invasive dental procedure over the course of the study. Furthermore, due to concerns regarding risk of vertebral fractures after discontinuation of denosumab, we will exclude patients with prevalent vertebral fractures and all patients must agree to referral to a bone disease specialist at the completion of their participation in this study. Our inclusion and exclusion criteria are:

Inclusion criteria

1. Men and women
2. All race-ethnicities
3. Age ≥ 18 years
4. ≥ 12-months after kidney transplantation (living or deceased donor recipient)
5. Stable allograft function over the previous year defined as:
    a. No rejections
    b. No more than a 15% decline in GFR over the prior year
6. Allograft GFR ≥ 30 mL/minute/1.73 m^2 (MDRD or CKD-EPI per local lab reporting)
7. 25OHD ≥ 30 ng/mL (determined at screening visit)
8. Serum calcium ≥ 9.0 mg/dL (determined at screening visit)
9. T-Score at the spine including and between -1.0 and -3.5 (determined at screening visit) and/or t-score at all other skeletal sites ≤ -1.0
10. Must have had a routine dental exam within 12-months of study recruitment
11. Must agree to continue with routine dental exams over the course of the study
12. Has not undergone an invasive dental procedure (i.e., tooth extraction, dental implants, oral surgery) within ≤ 3-months of recruitment
13. Must agree to referral to metabolic bone disease specialist at the end of the study
14. Women of child bearing potential must be willing to use one form of effective contraception over the course of the study

Exclusion Criteria

1. Allograft GFR < 30 mL/minute/1.73 m^2 (MDRD or CKD-EPI per local lab reporting)
2. Within 24-months of starting renal replacement therapy
3. Prevalent or occult vertebral fractures
4. History of post-transplantation non-basal cell carcinoma cancers within 5-years of enrollment and not in remission
5. Non-ambulatory
6. Malignancy requiring chemotherapy or metastatic to bone within 5-years of enrollment and not in remission
7. Non-transplant related metabolic bone diseases that alter bone mineral density, including but not limited to Primary hyperparathyroidism, Paget's, Osteogenesis Imperfecta
8. Within one-year of parathyroidectomy
9. Untreated hyperthyroidism for 6-months or longer
10. Untreated hypothyroidism for 6-months of longer
11. Medical diseases (end stage liver, lung or heart, intestinal malabsorption)
12. Use within the prior year of bisphosphonates, teriparatide, selective estrogen receptor modulators, testosterone, estrogen in the form of hormone replacement therapy*, denosumab, abaloparatide, calcitonin, and romosozumab
13. Allergy to components within the denosumab preparation or to denosumab
14. Weight > 300 pounds
15. PTH > 450 pg /mL
16. Will undergo an invasive dental procedure (i.e., tooth extraction, dental implants, oral surgery) within the next 12-months
17. Pregnant
18. Planned pregnancy during the course of the study

*OCPs are not exclusionary

D.2.2. Screening and Evaluation

D.2.2.a. **Pregnancy:** All pre-menopausal women will undergo a urine pregnancy test prior to receiving study drug, vertebral X-rays and BMD testing, and at the 12-month study visit.

D.2.2.b. Vertebral fractures: All patients will undergo screening thoracic and lumbar spine lateral X-rays to assess for occult vertebral fractures. Any thoracic and lumbar spine lateral X-ray results that were obtained within 12-months of the screening visit may be used.

D.2.2.c. Lumbar spine T-Score between -1.0 and -3.5: A screening BMD will be obtained if a DXA from within the 5-years prior to recruitment is not available. Any screening DXA that was obtained within 3-months of the baseline visit can be used as the baseline DXA as long as it was obtained on the same DXA machine as the one that will be used for this protocol. Any screening DXA that is obtained during the screening visit will also be used as the baseline DXA to ensure that patients are not exposed to additional radiation.

D.2.2.d. Calcium and 25OHD assessment: All participants will undergo screening of serum calcium and 25-hydroxy vitamin D to assess for risk of denosumab induced hypocalcemia. Prior to enrollment, all patients will need to have 25OHD levels ≥30 ng/mL and serum calcium levels ≥9 mg/dL. Lab results that were obtained within 3 weeks of the screening visit may be used. If 25OHD levels of < 30 ng/mL and serum calcium levels < 9 mg/dL prior to enrollment, they will be given the following supplements:

- Vitamin D
    - Vitamin D: 25OHD 20-30 ng/mL - Ergocalciferol 50,000 IU daily x 4 days
    - Vitamin D: 25OHD < 20 ng/mL - Ergocalciferol 50,000 IU daily x 8 days
- Calcium: Calcium carbonate 500 mg PO daily x 7 days

All participants will be rescreened for serum calcium and 25-hydroxy vitamin D labs after the supplementation period is complete. Participants will be allowed one rescreen visit.

D.2.3. Enrollment and Randomization: Each study site will randomize 20 participants and all analyses will be adjusted for study site (see **Section D.6.3.**). After recruits have passed screening and evaluation, the research teams at all study sites will redact and upload all source documentation of the screening visit into the REDCap file repository labeled with the assigned study code. If the DXA being used for the screen was from the patient's prior medical history, a copy of this record should be obtained and a signed medical record release form should be included per institutional policy. They will also fill out the "Screen Eligibility Form", which will notify Dr. Nickolas via email. Dr. Nickolas will review the screening documents and sign off on the "Screen Eligibility Form" via electronic signature. Screening documents should include, but is not limited to, DXA results, spine x-ray results, laboratory results, pregnancy test results, and dental exam confirmation. He will also email the research team to notify them of the approval before randomization can occur. The non-CUIMC sites will notify the study statistician by email to release blinded confirmation of randomization to local research study staff and the unblinded treatment assignment to the local research pharmacy.

The CUIMC research pharmacy will randomize enrolled participants using a randomization scheme prepared for this site. At the CUIMC site only, randomization will be 50% denosumab and 50% placebo since HR-pQCT imaging will occur only at CUIMC and our goal is to perform HR-pQCT imaging in 10 subjects on denosumab and 10 subjects on placebo. Therefore, in order to achieve the target group assignment of 40/20 denosumab/placebo for the total study, the randomization algorithm for the non-CUIMC sites will be adjusted to 3:1 denosumab:placebo. As described in **section D.6.3.**, statistical analyses will be adjusted for study site to mitigate possible effects of bias by recruitment center. The investigators and clinical research coordinators will be blinded to treatment assignment for the duration of the study. Due to the uneven distribution of treatment randomization (i.e. 40/20), it will be impossible to blind the statistician to randomization assignment. However, for reporting to the DSMB, the statistician will report adverse events only by group assignment (e.g., group A and B), but will not unblind the investigators or the

DSMB to treatment group (i.e., active drug or placebo). The DSMB will be permitted to ask for study unblinding only for specific circumstances as outlined below in the section **E.3.a.The Data Safety and Monitoring Board**.

D.2.3.a. Cholecalciferol and Calcium Supplementation: After enrollment and randomization and prior to starting study drug, all participants will be started on cholecalciferol 1,000 IU daily and calcium carbonate 500 mg daily to prevent denosumab induced hypocalcemia. If a patient is already taking vitamin D supplements prior to enrollment and their laboratory levels are at target, their vitamin D regimen will remain the same. If a patient is already taking calcium supplements prior to enrollment, they will be asked to switch to the regimen according to the study protocol. Additionally, patients whose calcium levels are over the upper range of normal (based on institutional standards) will not be placed on any calcium supplements. Subjects are required to bring their supplements to the 6- and 12-month visits to ensure and verify compliance. The research team will count the number of pills and document the number of pills taken since the last visit in the appropriate case report form. Management of post-injection hypocalcemia will be reviewed by the independent medical monitor is outlined below in the section **E.3.c.i. Management of hypocalcemia**.

D.2.4. Study Treatment (see **Table 2**)**:** After enrollment and randomization, **Study Treatment** phase will begin. During this phase, in-office visits will occur every 6 months and at that time participants will complete questionnaires outlined in detail in **Section D.7. Database and Clinical Research Data Collection Forms.** These forms pertain to medical, transplant and fracture history, dietary assessment and frailty/physical activity, kidney function, and compliance with study medications. There can be up to a 7 day window between the Baseline visit and the study drug administration to allow for review of the bone imaging results. *Safety labs will be obtained*: a renal panel will be obtained in the outpatient setting at the following time points: both denosumab injection visits, two-weeks after each denosumab injection (weeks 2 and 25, respectively) to assess for hypocalcemia, and one-week prior to the second denosumab injection (week 23) to ensure that serum calcium is ≥ 9.0 mg/dL. If hypocalcemia is present after an injection, we will proceed as outlined in the section **E.3.c.i. Management of hypocalcemia**. If calcium levels prior to the second injection are ≤ 9.0 mg/dL, we will administer calcium supplementation as outlined in section **D.2.2.d. Calcium and 25OHD assessment**.

Remote Visits: Remote visits using IRB-approved methods are permitted for data collection of medical history, concomitant medications, adverse events, physical activity questionnaire, and dietary questionnaire. Patients will be provided a copy of the questionnaires in person or digitally to facilitate the visit.

D.2.4.a. Compliance: Treatment will be administered by study personnel as a SQ injection every 6-months. Thus, compliance will be directly observed and we expect that all patients retained in the study will have 100% compliance unless they drop-out or experience a treatment-related adverse event.

D.2.4.b. Study drug self-injection: Due to circumstances surrounding COVID-19, patients will have an option to do self-injection of the study drug to limit the amount of time spent on the medical campus. The research pharmacy will continue to blind the study drug in preparation for shipment. The study team will make arrangements with a carrier to provide temperature-controlled transportation of the study drug and communicate with the patient to ensure that the patient is home to receive the shipment. The study doctor will then arrange a video visit with the patient for the injection. Follow-up with the patient after the self-injection will occur as per regular protocol in order to monitor for adverse events.

D.2.4.c. Drop-outs: We are inflating our sample size by 10% to account for drop-out and loss to follow-up (see **D.6.3. Statistical plan and Sample Size Justification for both Aims 1 and 2)**. Since the population being studied represents a healthy subset of kidney transplant recipients, we expect that post-transplant survival rates will exceed 90%, and we do not anticipate meaningful loss to follow-up due to

death. A 10% drop-out rate will not compromise our ability to obtain meaningful statistical results on all our planned outcomes. For our primary outcome of the percent change in BMD by DXA, we will be able to obtain meaningful statistical results if we have up to 65% and 35% drop-out in the denosumab and placebo drug groups respectively.

D.2.4.d. Participants will be withdrawn from continuation of drug administration after the first injection due to the adverse events itemized below. Should a subject meet withdrawal criteria, all study sites will update the "Enrollment/Study Completion Form" in REDCap with the final study status and note the withdrawal criteria. The non-CUIMC sites will also upload redacted source documentation into REDCap and notify Dr. Nickolas via email. All study sites will maintain the source documentation in the participant's study binder. These participants will be considered "Passive Participants", meaning at the time of withdrawal, only study medication will be stopped, and the participant will continue to be followed to provide endpoint data at 12-months. These data will be adjusted in analyses to reflect that they were obtained after a single injection of either study drug or placebo (See **Section D.6.3**). The following events will be used to determine need for withdrawal:

- De novo hypersensitivity to the study drug
- Refractory hypocalcemia
- Hypocalcemia not due to study drug and determined to be of unclear etiology
- Refractory eczema or dermatitis
- Refractory diarrhea
- Multiple or refractory urinary tract infections
- Pyelonephritis
- The development of a serious infection that is not responsive to standard antibiotic therapy
- Pregnancy
- The need for emergent invasive dental procedures
- Allograft rejection
- Osteonecrosis of the jaw (ONJ)
- Atypical femoral fractures
- BMD loss by DXA ≥ 7% at any skeletal site

D.2.4.e. Study Stoppage: The study will be stopped at the discretion of the Data Safety and Monitoring Board as described in **Section E.3.a.** If the number of serious adverse events, itemized in **D.2.4.d** exceeds 10% (6 participants) and a ratio of 2:1 or greater in the denosumab vs. placebo groups, the DSMB will request an *ad hoc* audit to evaluate the relatedness between the adverse events and the study. If the *ad hoc* audit identifies concerns, the DSMB may recommend study stoppage.

D.2.5. Post-Treatment Follow-up and Bone Care: After completion of the **Study Treatment** phase, study participants will be contacted via telephone every three months for 12-months after study drug is completed (15-, 18-, 21-, and 24-month visit) to assess for post-treatment adverse events (see **D.7. 13 and 14**). Furthermore, all participants will be scheduled to see a metabolic bone disease specialist within one-month of completing the **Study Treatment** phase.

D.3. Aims 1 and 2. Methods

Data will be collected as per **Table 2**. At baseline, 6-months, and end of treatment:

- Bone mineral density at the spine, hip and forearm by DXA in all patients
- HR-pQCT (Scanco XtremeCT2, resolution 61 μm^3) to measure cortical and trabecular volumetric BMD and microarchitecture and trabecular and whole bone biomechanical competence (micro finite element analysis [μFEA]) at the ultradistal radius and tibia in all patients recruited at CUIMC
- Blood for research assays will be collected and stored at -80ºC, at baseline (pre-treatment) and after 6- and 12-months of treatment (**Table 2:** calcium, phosphorus, PTH, vitamin D metabolites, bone

formation (bone specific alkaline phosphatase [BSAP], procollagen type-1 N-terminal propeptide [P1NP]), resorption (C-Telopeptide [CTX]) markers, FGF-23 and sclerostin, and other markers of skeletal health.

- Blood for screen and safety labs
- Urine for pregnancy testing at screen, baseline/randomization, 6-, and 12-months
- Questionnaires for dietary intake of calcium/vitamin D, physical activity, and frailty
- Research staff will maintain a concomitant medication log throughout the study beginning with the baseline visit to assess for exclusion criteria and potential drug interactions. At the start of the 6- and 12-month visits, the research staff will reconcile any previous medications in the log and document new medications.

Table 2: Study Procedures for Aims 1 and 2

	Recruit-ment	Screening	Base-line	Study Drug Admin-istra-tion	2-weeks post Drug admin-istra-tion	1-week pre 6-mo	6-mo	2-weeks post 6-mo	12-mo	15-, 18-, 21-, 24-mo
Scheduling Window		(- 3 weeks from Baseline Visit)		(+ 7 days from Base-line)	(+/- 3 days)	(- 2 weeks)	(+/- 2 weeks)	(+/- 3 days)	(+/- 2 weeks)	
Review of Eligibility Criteria	**X**									
Screening Tests: ICF, Spine X-rays, DXA, renal panel, calcium, and 25OHD and pregnancy testing		**X**								
Review of interim history, adverse events, incident fracture reporting, vital sign measurement			**X**				**X**		**X**	
Physical activity/frailty questionnaires			**X**						**X**	
Block Calcium and Vitamin D Screener			**X**							
Drug administration			**X**	**X**			**X**			
Calcium/Vitamin D pill dispensation			**X**				**X**			
Calcium/Vitamin D pill count							**X**		**X**	
Concomitant Medications Reconciliation			**X**				**X**		**X**	
Renal panel		**X**	**X**		**X**	**X**	**X**	**X**		
Urine Pregnancy test		**X**	**X**				**X**		**X**	
DXA: LS, TH, FN, Forearm (All sites)			**X**				**X**		**X**	
HR-pQCT: radius and tibia (CUIMC site only)			**X**				**X**		**X**	

Research Biochemistries: see Table 3			**X**				**X**		**X**	
Adverse Event Ascertainment			**X**		**X**	**X**	**X**	**X**	**X**	**X**
Follow-up Phone Call										**X**

D.3.1. Imaging Methods for Measurement of Bone Mass, Microarchitecture, and Mechanical Competence

D.3.1.a. Image Acquisition, Machine Calibration and QA/QC, Mechanical Analysis of Skeletal Imaging and Image Data Storage

D.3.1.a. i. Sanchita Agarwal, MS at CUIMC is an imaging and image analysis expert. She will coordinate scan acquisitions of DXA and HR-pQCT as well as QA/QC, image acquisition protocols, DXA cross-calibration, and will perform analyses of HR-pQCT datasets. Prior to the enrollment of the first patient, Ms Agarwal will conduct a teleconference with the site PIs and CRCs to review image acquisition protocols, which include but is not limited to scanning procedures, review of skeletal sites to be imaged, development and review of the image acquisition form, protocol for the distribution of the circulating phantom, and procedures for transfer of imaging data for review and analysis and long-term storage of image files. Ms Agarwal will conduct teleconferences every 6-months after enrollment of the first patient to review imaging data, QA/QC, challenges with image acquisition and coordination of data transfer to CUIMC.

D.3.1.a.ii. Storage of DXA and HR-pQCT Imaging Data

- **DXA** image data will be stored locally, at each study site, on DXA machines for scanner analysis. Post analysis all image files will be stored, identified with only a study ID number, on a secure shared drive at CUIMC indefinitely. DXA image files will be transferred to CUIMC by secure FTP server. Quantitative analytical output of DXA image analyses, including but not limited to bone mineral content, area, density and T- and Z-Scores will be uploaded in CSV format and stored both on a secure shared server (source document) and in REDCap. We will also upload to REDCap the imaging results summary that is provided to the participants in a PDF format.
- **HR-pQCT** imaging will occur only at CUIMC and data will be stored locally on the scanner for analysis. Post-analysis all image data will be backed-up on a secure server as well as storage tapes, identified with only a study ID number, at CUIMC indefinitely. Quantitative analytical data of HR-pQCT images including but not limited to cortical and trabecular geometry, volumetric density, microarchitecture and finite element analysis will be uploaded in excel format and stored both on a secure shared server (source document) and in REDCap.

D.3.1.b. DXA Patients at all study sites will undergo measurement of areal BMD at baseline, 6- and 12-months on a study-specific designated DXA machine that will be used throughout the whole study. Imaging procedures will occur after the urine pregnancy test has been performed and prior to study drug administration. We will obtain BMD at the spine (L1-4, AP), proximal femur, and forearm. *CUIMC*: 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. For DXA machine cross-calibration, a traveling phantom (European spine and forearm phantom) will be circulated across sites prior to study start-up and study completion. All cross-calibration data will be recorded and accounted for at time of analysis.

D.3.1.c. HR-pQCT Only patients recruited at CUIMC will undergo measurement of cortical and trabecular bone microarchitecture and biomechanical competence. We will use the XtremeCT-II (Scanco Medical

AG, Brüttisellen, Switzerland, isotropic voxel size 61 µm). Patients will undergo imaging at baseline, 6- and 12-months. Methods and QC are described in our publications[8,9]. In brief, HR-pQCT imaging will be obtained at the non-dominant radius and tibia. The dominant limb will be scanned if there is a fracture or dialysis access at the non-dominant limb. Three regions of interest will be scanned: (1) fixed offset ROI; (2) relative offset ROI; and (3) a predominant cortical ROI at the 30% site. Precision: cortical and trabecular volumetric BMD=0.9%±7.1% and 2.3±3.5%, respectively. Motion grading (score of 0 to 5) is done for all scans at the time of image acquisition and scans with score > 3 will be repeated if the participant agrees.

D.3.1.d. 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 compression is simulated equaling 1% strain using a homogenous Young's modulus if 6829 Mpa and Poisson's ratio of 0.3 to estimate stiffness. This compression is applied on the distal end of the bone segment. Failure load will be estimated based on the criterion by Pistoia et al[10].

D.4. Questionnaires
D.4.1. Grip Strength will be used to measure frailty
D.4.2. The Modified Baecke Questionnaire will be used to measure physical activity
D.4.3. The Block Calcium/Vitamin D Screener provided by NutritionQuest will be used to measure dietary intake of calcium and vitamin D

D.5. Bone Biomarker Analysis
All assays will be done in the Bone Marker Lab at CUIMC. Every effort will be made to obtain 30 mL of blood after an 8-hour fast and in the morning. Patients are permitted to take their medications prior to the morning blood draw. If patients are taking insulin or hypoglycemic, they will hold those medications until after the blood draw and after eating. All blood will be stored locally at each study site at -80°C. At study end, the sera will be shipped to CUIMC and batch assayed. Biochemical assays are itemized in **Table 3**.

Table 3: Biochemical Assays

Assays	Method	Type	Assay Volume	Manufacturer
Phosphate	Automated (integra)	Serum	200ul	Roche
Calcium	Automated (integra)	Serum		Roche
Vitamin D metabolites (25OHD; 1,25OHD; 1,24OHD)	LCMS	Serum	200ul	
Intact Parathyroid Hormone (PTH)	RIA	EDTA plasma	200ulx2	Scantibodies
Bone Specific Alkaline Phosphatase (BSAP)	ELISA	Serum, heparin plasma	20ulx2	Quidel
Procollagen Type-1 N-Terminal Propeptide (P1NP)	RIA	Serum	50ulx2	Orion Diagnostica (Espoo Finland)
Carboxy-Terminal Cross-linked Telopeptide of Type 1 Collagen (CTX)	ELISA	Serum, EDTA plasma	50ulx2	Immunodiagnostic Systems, Scottsdale AZ, USA
Tartrate Resistant Acid Phosphatase 5b (Trap-5b)	ELISA	Serum/ EDTA plasma	100ulx2	Immunodiagnostic Systems, Scottsdale AZ, USA
FGF-23*	ELISA	EDTA plasma	100ulx2	Immunodiagnostic Systems, Scottsdale AZ, USA
Sclerostin	ELISA	Serum or Plasma	20ulx2	Tedco Medical (Switzerland)

*FGF23: intact and C-term

D.6. Outcomes, Statistical Plan and Sample Size Justification

D.6.1. The primary outcome for Aim 1 is the percent change from baseline in DXA spine BMD at the 12-month endpoint. Secondary outcomes include the raw value change in DXA BMD at the spine and the percent and raw value change in DXA BMD at the hip and forearm at the 12-month endpoint.

D.6.2. The primary outcome for Aim 2 is the percent change in HR-pQCT stiffness at the distal radius and tibia at the 12-month endpoint. Secondary endpoints include the percent and raw value change from baseline in HR-pQCT tibia and radius cortical volumetric density, thickness and porosity, and trabecular volumetric density, number and thickness at the 12-month endpoint.

D.6.3. Statistical plan and Sample Size Justification for both Aims 1 and 2, between group differences in the percent change from baseline will be assessed with ANCOVA with the baseline value of the outcome included as a continuous covariate, and with independent T-tests for the between group difference in the raw value change from baseline. No adjustment for multiple testing will be made for DXA measures while a permutation step-down p-value adjustment will be made for HR-pQCT measures within the tibia and radius vectors. Participants who have study drug withheld due to adverse events will be followed to provide endpoint data at 12-months, and these data will be adjusted in analyses to reflect the proportion of the study in which they received treatment (i.e., 1 for two injections and 0.5 for a single injection). All models will block on recruitment site.

D.6.3.a. For the lumbar spine, according to Bonani et al [5], at 12 months the Control group had a decline in LS BMD by 0.500%, 95% CI -1.8% to 0.9% and the denosumab group had an increase in LS BMD by 4.6%, 95% CI 3.3% to 5.9%. With n= 44 and n=46 in two groups, respectively, that translates to -0.5000 $\pm$ 4.5227 and +4.6 $\pm$ 4.530, again respectively. This difference is a standardized effect size of 1.1277 which requires 13 subjects / group for 80% power, 5% alpha or 19 subjects / group for 80% power, 1% alpha. With 40 denosumab and 20 placebo subjects, the harmonic mean of the sample size is 27, which would provide 80% power, 5% alpha to detect an effect of denosumab equal to 3.02% or larger; or provide 80% power, 1% alpha to detect an effect of denosumab equal to 3.70% or larger.

D.6.3.b. For the total hip, Bonani et al [5], at 12 months the control group had an increase in TH BMD by 0.4%, 95% CI = -0.8% to 1.7% and the denosumab group had an increase in TH BMD by 2.3%, 95% CI = 1.1% to 3.5%. With n= 44 and n=46 in two groups, respectively, that translates to -0.400 $\pm$ 0.8186 and +2.3 $\pm$ 4.103, again respectively. This difference is a standardized effect size of 2.32 which requires 4 subjects / group for 80% power, 5% alpha or 5 subjects / group for 80% power and 1% alpha. With 40 denosumab and 20 placebo subjects, the harmonic mean of the sample sizes is 27, which would provide 80% power, 5% alpha to detect an effect of denosumab at the hip equal to 1.036% or larger; or provide 80% power, 1% alpha to detect an effect of denosumab at the hip equal to 1.159% or larger.

D.6.3.c. Calculations for spine and hip assume that the mean of the placebo group is the same as that reported by Bonani et al [5] and that the variability in the groups also remains as reported by Bonani et al [5]. Data from Brunova et al[11], a study conducted in prevalent solid organ transplant recipients that also included recipients of a kidney transplant only, support the estimates of

Table 4	Bonani		Brunova	
	Control	Denosumab	Denosumab	
LS % change 12	-0.5 $\pm$ 4.52	4.6 $\pm$ 4.45		
LS % change 20mo			10.1 $\pm$ 5.90	*kidney transplant only
TH % change 12mo	0.4 $\pm$ 4.18	2.3 $\pm$ 4.11		
TH % change 20mo			10.4 $\pm$ 8.3	*kidney transplant only

changes in LS and Hip BMD from Bonani et al (See **Table 4**).

D.6.3.d. For stiffness at the radius and tibia by HR-pQCT. For HR-pQCT we will enroll 10 participants per group and our primary outcome is the percent change in stiffness at the radius and tibia at the 12-month endpoint. This sample size permits detection of a 1.32 SD difference in stiffness at the radius and tibia with alpha 0.05 / beta 0.80. Based on Bonani et al [6], the mean percent difference ± SD in stiffness at the radius and tibia over the first 12-months of kidney transplantation was 2.9% ± 3.3% and 5.6% ± 1.9%. Therefore, we expect to be able to detect a between-group difference in stiffness over 12-months of treatment.

D.7. Database, Data Entry, and Clinical Research Data Collection Forms

- This project will use REDCap at all sites. A centralized REDCap database will be developed by CUIMC and used at all sites.
- CUIMC and Northwestern will use REDCap as their source documentation for CRFs, excluding image files for DXA and HR-pQCT, NutritionQuest Forms, and laboratory results.
- NorthShore will maintain paper documents as their source documentation and will copy into REDCap.
- Study visit data will be entered within one-month of the study visit completion unless otherwise stated
    - A copy of the Block Calcium/Vitamin D screener will be uploaded to the file repository in RED-Cap, and the physical copy will be sent to NutritionQuest for scoring at the end of the study
- Adverse event data will be entered within 48-hours of the study visit completion unless otherwise stated

The following CRFs will be developed in REDCap for data collection:

1) Screening form
2) Eligibility / Randomization form to document result of screening and randomization process
3) Demographic form
4) Baseline visit form (visit 1)
    a. Visit 1 procedure checklist
    b. Medical history
    c. Transplant history and allograft function
    d. Family history
    e. Social history
    f. Fracture history
    g. Medication history
    h. Block Calcium and Vitamin D Screener
    i. Modified Baecke physical activity
    j. Documentation of study drug injection (study drug compliance)
    k. Blood sample tracking
5) Concomitant medication reconciliation
6) Safety lab tracking form: Two-week renal panel
7) Safety lab tracking form: Twenty-three-week renal panel
8) 6 Month visit form (visit 2)
    a. Visit 2 procedure checklist
    b. Updated medical history
    c. Updated transplant history and allograft function
    d. Updated medication history
    e. Documentation of study drug injection (study drug compliance)
    f. Calcium and Vitamin D pill count
    g. Blood sample tracking
9) Safety lab tracking form: Twenty-five-week renal panel
10) 12 Month visit form (visit 3)

a. Visit 3 procedure checklist
b. Updated medical history
c. Updated transplant history and allograft function
d. Updated medication history
e. Modified Baecke physical activity
f. Documentation of study drug injection (study drug compliance)
g. Calcium and Vitamin D pill count
h. Blood sample tracking

11) 3-Month Follow-up Tracking
    a. 15 Months Follow-up
        i. Confirmation of post-treatment bone care
        ii. Adverse and serious adverse event assessment (see **D7. 13 and 14**)
    b. 18 Months Follow-up
        i. Confirmation of post-treatment bone care
        ii. Adverse and serious adverse event assessment (see **D7. 13 and 14**)
    c. 21 Months Follow-up
        i. Confirmation of post-treatment bone care
        ii. Adverse and serious adverse event assessment (see **D7. 13 and 14**)
    d. 24 Months Follow-up
        i. Confirmation of post-treatment bone care
        ii. Adverse and serious adverse event assessment (see **D7. 13 and 14**)
12) Termination form
    a. Participants who complete the study
    b. Participants who terminate study procedures early and the reason for termination
13) Adverse event tracking
    a. Hypocalcemia
    b. Incident fractures
    c. Urinary tract infections
    d. Non-urinary tract infections
    e. Refractory Diarrhea
    f. Incident cancers
    g. Dermatitis and Eczema
    h. Transplant rejection episodes
    i. Hypersensitivity
    j. Pyelonephritis
    k. Osteonecrosis of the jaw (ONJ)
    l. Atypical femoral fractures
    m. Serious infection leading to hospitalization
    n. Other expected AEs
    o. Unexpected AEs
14) Serious adverse event forms
    a. Will use MED-WATCH to monitor/document SAEs
15) Imaging (DXA and HR-pQCT) and biochemical data forms

E. HUMAN SUBJECTS PROTECTIONS

E.1. Adverse Events and Serious Adverse Events

E.1.a. Definition of Adverse Event (AE)

- **Adverse event:** any untoward or unfavorable medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related

E.1.b. Definition of Serious Adverse Event (SAE)

- **Serious adverse event:** an adverse event or suspected adverse reaction is considered serious if, in the view of the investigator, it results in any of the following outcomes:
  - Death
  - Life-threatening adverse event
  - Inpatient hospitalization or prolongation of existing hospitalization
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
  - A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. These events should also be treated like an SAE.

E.1.c. Classification of an Adverse Event

E.1.c.i. Severity:

- **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

E.1.c.ii. Relatedness:

- **Definitely Related** – 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.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in

which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

E.1.c.iii. Expectedness
- **Expected adverse event:** An event that is listed in the investigator brochure and/or occurs at the expected incidence rate or severity that has been observed in the prior literature
- **Unexpected adverse event**: An event that is not listed in the investigator brochure or occurs at an incidence rate and/or severity that has not been observed in the prior literature

E.1.d. Adverse Event Reporting and Timeline: AEs will be collected continuously throughout this study and will occur at all study visits, including but not limited to visits for study drug injections and safety and pre-injection labs. All AEs will be captured by the research team at all sites on the appropriate case report form (CRF) and then documented in the REDCap database within 3 days of the research team becoming aware of its occurrence. Information to be collected includes event description, time of onset, assessment of severity, and relationship to the study drug or to study procedures (e.g., imaging, supplements). All AEs occurring while on study must be documented appropriately regardless of relationship. At each in-person study visit, the research staff will inquire about the occurrence of AE/SAEs since the previous visit and update any unresolved AEs. All AEs will be followed to adequate resolution.

E.1.e. Serious Adverse Event Reporting and Timeframe

Within 24-hours, the study investigators at all sites will report to the study sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.
- All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.
- Any unexpected fatal or life-threatening suspected adverse reaction will be reported to the FDA and all participating investigators as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information.
- Both any non-life threatening or non-fatal serious adverse reaction and any potential serious risks, identified from other clinical trials or other sources, will be reported to the FDA and all participating investigators in an IND safety report as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.
- Reporting to the FDA will occur via MED-WATCH

E.2. Unanticipated Problems

E.2.a. Definition of Unanticipated Problems (UP)
- **Unanticipated Problems** will be defined as any incident, experience, or outcome involving risk to subjects or others in any human subjects research that meet all of the following criteria:
    - Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the IRB-approved protocol and informed consent form, and (b) the characteristics of the subject population being studied.
    - Related or possibly related to participation in such research (i.e. there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in such research); and
    - Suggests that the research places the subject or others at a greater risk of harm (physical, psychologic, economic, or social) than was previously known or recognized.

E.2.b. Unanticipated Problem Reporting and Frequency

E.2.b.i. The study investigators at all sites will report UPs according to their own institutional policies. In addition, the study investigators at non-CUIMC sites must report any UPs occurring at their sites to the study sponsor within 48-hours. The study sponsor shall adhere to the following workflow as applicable:

- Submit the UP report to the CUIMC IRB within 7 days of either its occurrence or acquiring knowledge of its occurrence
- Submit a MED-WATCH form to the FDA by following the reporting guidelines detailed in **Section E.1.e.**
- Disseminate the UP report to the study investigators at non-CUIMC sites
- Submit a report to Amgen according to **Section E.5. Table 5**

E.2.b.ii. The UP report will include the following information:

- Protocol identifying information: Protocol title and number, PI's name, and the IRB project number
- A detailed description of the event, incident, or outcome
- An explanation of the basis for determining that the event, incident, or outcome represents an UP
- A description of any changes to the protocol or other corrective actions that are proposed in response to the UP

E.3. Events of Special Interest

E.3.a. Fractures: Incident fractures occurring during the study will result in referral to a metabolic bone disease specialist for management options. If the participant and the referring bone disease specialist request unblinding and clinical treatment.

E.3.b. Infections, Cancers and Cardiovascular Events: Treatment of infections, cancers and cardiovascular events will be deferred to the participant's nephrologist.

E.3.c. Hypocalcemia: Defined as an albumin adjusted serum calcium level less than the lower limit of normal for the core lab at each center. Serum calcium and albumin will be measured 1-week before and 2-weeks after each injection by local labs. At enrollment and prior to the first injection, all patients will be placed on calcium carbonate and cholecalciferol. Patients with post-injection hypocalcemia will be managed by the following protocol:

E.3.c.i. Management of hypocalcemia

1. The medical monitor at each recruitment site will review the corrected serum calcium from the safety labs obtained two-weeks after each injection and in the occurrence of a hypocalcemic event will send an email to the site PI and CRC with notification. The site PI and CRC will manage hypocalcemia with calcium and calcitriol based on the following dosing algorithm.
2. Drop in corrected serum calcium WNL of the reference range
    a. No dose change
3. Drop in serum calcium < LLN of the reference range but ≥ 7.5 mg/dL without symptoms of hypocalcemia
    a. Increase calcium carbonate to 500 mg twice daily and recheck in one week
        i. If calcium has normalized: no dose change to calcium supplement
        ii. If calcium remains < LLN but ≥ 7.5 mg/dL, increase calcium carbonate to 500 mg three-times daily and recheck in one week
        iii. If calcium remains < LLN but ≥ 7.5 mg/dL, start calcitriol 0.25 mcg three-times weekly
4. Drop in serum calcium < 7.5 mg/dL or below the reference range with symptoms of hypocalcemia
    a. Increase calcium carbonate to 500 mg three-times daily and start calcitriol 0.25 mcg daily and recheck in one week
        i. If calcium has normalized: no dose changes
        ii. If calcium level < LLN but ≥ 7.5 mg/dL without symptoms, increase calcitriol to 0.25 mcg

twice daily and recheck in one week

iii. If calcium level < 7.5 mg/dL or below the reference range with symptoms of hypocalcemia, increase calcium carbonate to 1,000 mg three-times daily and recheck in one week

iv. If calcium level < LLN but ≥ 7.5 mg/dL without symptoms, proceed as in 4.a.ii

5. **Hypocalcemia occurring > 2 weeks from study drug administration and from non-study safety labs:** The research teams at all sites will adjudicate whether or not the hypocalcemic event is due to study drug administration. If the event is due to the study drug, the event will be managed as per above. If the event is not due to the study drug, the etiology will need to be either determined and corrected before study drug is administered, or the participant will be withdrawn from study drug administration and will be followed as a passive participant per **Section D.2.4.d.**

E.4. Safety Oversight

E.4.a. The Data Safety and Monitoring Board (DSMB)

- An independent DSMB will be established to monitor patient safety and to evaluate the progress of the study and adverse events related to study participation. The DSMB will be comprised of a transplant nephrologist, a bone disease specialist and a statistician. Our DSMB members include:
  - Peter Reese MD MSCE, Division of Nephrology, The University of Pennsylvania, Philadelphia, PA
  - Bart Clarke MD, Division of Endocrinology, Mayo Clinic, Rochester, MN
  - Shoukoufeh Khalatbari MS, Biostatistics Program, Michigan Institute for Clinical and Health Research, University of Michigan, Ann Arbor, MI
- The DSMB will meet prior to study initiation to ensure that study procedures are compliant with IRB and HIPAA guidelines, to establish reporting guidelines of adverse events to the PIs and sponsor, and to recommend protocol revisions.
- After enrollment of the first study participant, the DSMB will meet every six months, or on an as needed basis, during the study's duration to review enrollment rates, protocol adherence, and monitoring and reporting of adverse events.
- Over the course of the study, the DSMB may ask for unblinding, a change of the protocol and/or consent of the study, or study stoppage based on their review of the data.
- The DSMB will make decisions about unblinding, a change of the protocol and/or consent of the study, or study stoppage if the number of adverse or serious adverse events exceeds 10% (6 participants) and a ratio of 2:1 or greater in the denosumab vs. placebo groups, or vice versa, although the DSMB's decisions will not be restricted to those specific circumstances.
- As a potential step prior to recommending study stoppage or changes to the study, the DSMB may consider initiating an ad hoc audit conducted by the CUIMC departmental QA monitor. The audit will focus on relationships between the relatedness of adverse events and study drug and procedures.
- The DSMB will be supplied the following data by the CUIMC PI, CRC, and statistician:
  - CONSORT Diagram
  - Data tables that summarize demographic and baseline clinical characteristics
  - Data quality tables that capture missing visits
  - Safety assessments of aggregate tables of adverse events and serious adverse events
  - Aggregate tables of clinical laboratory values
  - Listings of protocol deviations and violations
  - Summary of protocol changes since the prior DSMB meeting
- The DSMB will be responsible for reviewing and making recommendations based upon data pertaining to safety and adverse events. The recommendations of the DSMB will be as follows:
  - Continue without amendment
  - Continue with amendment

- Suspend enrollment
- Discontinue study

E.4.b. Independent Medical Monitor: An unblinded medical monitor at each recruitment site will review safety labs and make recommendations on management of hypocalcemia. The medical monitor will adhere to the following workflow:

- Review unblinded safety labs
- Report the adverse event (i.e. hypocalcemia) to the site investigator and local CRC and also to the study sponsor via email
  - The local CRC will update both the adverse event case report form and REDCap with the necessary information
- Report the actual safety lab values to the study statistician, who will compile a table that will be reported to the DSMB at the regular 6-month meetings

E.5. Study Site Monitoring:

- Remote and on-site monitoring visits will each occur at least once annually.
- All study documentation and source documents that is captured in paper format and recorded into a central REDCap EDC database has to be made available during study site monitoring visits.
- Recording of paper documentation into REDCap will be verified according to protocol.
- Non-CUIMC recruitment sites will be monitored remotely by the CUIMC Lead CRC prior to study initiation, after the completion of the first subject's baseline visit, and then annually after recruitment of the first patient until study completion.
  - On-site monitoring will occur once yearly.
  - Targeted review of data (< 100% data verification) through the REDCap database will occur at least once per year during the remote site monitoring visit, and total data verification will occur during the annual on-site monitoring visit.
- CUIMC recruitment site will be monitored on-site by a CUIMC departmental QA monitor prior to study initiation, after the completion of the first subject's baseline visit, and at least annually after recruitment of the first patient until study completion.

E.6. Safety Reporting to Amgen and Regulatory Authorities: We will comply with Amgen policies on safety reporting, as outlined in the Amgen Safety Reporting Guidelines (**Table 5**). Reports will be sent to Amgen through either fax (1-888-814-8653) or email (svc-ags-in-us@amgen.com) within the specified timeframe below.

Table 5: Safety Reporting Timeframe to Amgen	
Safety Data	**Timeframe for Submission to Amgen**
Suspected Unexpected Serious Adverse Reaction (SUSARs)	Sent to Amgen at time of regulatory submission
Pregnancy/Lactation	Within 10 calendar days of Sponsor awareness
Annual Safety Report (eg, EU Clinical Trial Directive [CTD] DSUR, and US IND Annual Report)	Annually
Other Aggregate Analyses (any report containing safety data generated during the course of a study	At time of ISS sponsor submission to any body governing research conduct (eg, RA, IRB, etc)

Final (End of Study Report, including): • Unblinding data for blinded studies • Reports of unauthorized use of a marketed product	At time of ISS sponsor submission to any body governing research conduct (eg, RA, IRB, etc) but not later than 1 calendar year of study completion

E.7. Institutional Review Board
IRB approval of the protocol and informed consent form/procedures at all study sites will be obtained prior to recruitment and all subjects will have ICF prior to any study procedures.

E.8. Incidental Findings (IF)
Skeletal imaging in this protocol involves X-rays of the thoracic and lumbar spine, DXA of the spine, hip and forearm and HR-pQCT of the distal radius and tibia. X-rays of the thoracic and lumbar spine are clinical scans used to identify vertebral fractures. DXA imaging is a clinical scan that is used for the diagnosis and monitoring of osteoporosis. HR-pQCT is a research scan that is used to assess microarchitectural aspects of bone quality; it is not used in the clinic and is not FDA approved for clinical use. As pertains to this protocol, according to CUIMC IRB policy, X-rays of the thoracic and lumbar spine require IF review.

E.8.a. Summary of IF's for spine X-ray imaging may include but are not limited to:
1. Vertebral fractures
2. Sclerotic lesions
3. Aortic aneurysms
4. Osteoarthritis
5. Scoliosis
6. Aortic calcifications
7. Osteophytes

E.8.b. Plan for Imaging Review. X-ray images will be reviewed by a radiologist per clinical standard and the results will be reported in the electronic health record.

E.8.c. IF Disclosure Plan: The results of all spine X-rays will be reviewed either verbally or by written communication with the study participant by the PI at each study site (CUIMC: Dr. Nickolas; Northwestern: Dr. Isakova; NorthShore: Dr. Sprague).

E.8.d. IF Classification: IF's will be classified as A or B and all Class A / B IF's will be reviewed verbally

E.8.d.i. **Class A IF:** These are life-threatening or severe. The likelihood of identifying a Class A IF by spine X-ray imaging is highly unlikely. If a Class A IF is identified, that information will be communicated immediately and directly to the study participant and their primary care provider.

E.8.d.ii. Class B IF: These are not necessarily immediately life threatening or severe, but are likely to be deemed by a subject to be important to his/her health. If a Class B IF is identified, that information will be communicated immediately and directly to the study participant and their primary care provider and/or nephrologist.

E.8.e. All IF's of Clinical Significances and the management of such findings will be documented in the research records for the study.

E.8.f. At the time of a continuing review, if an IF was noted during the previous approval period, the PI will provide the IRB with the following information:
1. The number of required Review Images

2. A list of the subject study numbers
3. The type of scan
4. The date of the scan
5. A description of the IF of Clinical Significance
6. The date of communication with the subject and the outcome, if known.

E.9. FDA IND Sponsor Responsibilities
E.9.a. The Sponsor-Investigator has completed the CUIMC IRB course TC0096: *FDA Requirements of Sponsor-Investigator Studies*

E.9.b. The Sponsor-Investigator agrees to comply with the FDA and IRB outlined responsibilities, including but not limited to

- Ensuring proper monitoring
- Ensuring the study is conducted in accordance with the protocol
- Reviewing ongoing investigations/evaluating safety and efficacy/reporting to the FDA and IRB
- Keeping and retaining records/documentation
- Submitting amendments, IND Safety Reports, and Annual reports to the FDA

E.10. 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 determine the effects of denosumab on your bone density". The study methods, namely the administration of denosumab; assessment of medical, dietary intake, fracture history, and physical activity; the evaluation by imaging modalities; and the collection of blood 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.

E.11. Potential Risks
The *potential risks* are related to *exposure to the study drug, vitamin D and calcium carbonate*, *loss of confidentiality, venipuncture, and radiation exposure* from DXA for measurement of bone density and HR-pQCT for measurement of volumetric density and microarchitectural parameters.

E.11.a. Loss of confidentiality: due to breaches in data security or deductive disclosure. To ensure that participants' confidentiality is not compromised. To minimize these risks, 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

based on REDCap, 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 lists for CUIMC, Northwestern and NorthShore will be kept on secure servers on their respective research shared access networks. All data containing PHI will be stored on password-protected encrypted computers 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 or parent/legal guardian as applicable. 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.

The methods in place for maintaining patient confidentiality will be explained to the subjects during the Informed Consent process. This will include registering all data under a subject specific alpha-numeric system and storing all records in password protected and encrypted files in the PIs' office. Participant data will be de-identified and entered into a password secured database and will be available only to investigators and key personnel directly involved in this research. All electronic data will be analyzed on password-protected, encrypted workstations. Only investigators directly involved in this research study will have access to subject identities. In addition, such data will be available to the IRB.

E.11.b. Venipuncture: The risks of venipuncture for blood drawing include pain, bleeding, bruising, infection and inflammation at the site. The risks of venipuncture will be minimized by the use of trained experienced professional staff to obtain all blood samples.

E.11.c. Radiation: The total expected radiation dose for the entire study will vary depending on whether the participant is undergoing HR-pQCT scanning in addition to DXA scanning (participants recruited at CUIMC will under HR-pQCT imaging). 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, HRpQCT, and study drug administration. Pregnant females will be excluded from the study at any time point in order to protect the unborn fetus. Radiation safety approval will be obtained prior to initiating recruitment at all centers as part of the IRB approval process.

E.11.c.i. Thoracic and Lumbar Spine X-rays: All recruits will undergo spine X-rays to assess for occult vertebral fractures. T- and L-spine X-rays each deliver 0.3 mSv of total body radiation. Therefore, over the course of this study, participants will be exposed to 0.6 mSv of total body radiation from spine X-rays.

E.10.c.ii. DXA: Scanning will occur at baseline, 6- and 12-months. A single DXA scan at the spine, hip, forearm and whole body using the Hologic QDR4500 in fast scan mode is associated with 0.01 mSv of total body radiation. Therefore, over the course of this study, participants will be exposed to 0.03 mSv of total body radiation from DXA.

E.11.c.iii. HR-pQCT: Participants enrolled at the CUIMC site will undergo bone quality imaging at the radius and tibia by HR-pQCT at baseline, 6- and 12-months in addition to DXA. The HR-pQCT scan region of interest will be at 3-sites: fixed, relative and a cortical bone proximal site. A single HR-pQCT scan delivers 0.03 mSv of total body radiation. Therefore, over the course of this study, participants will be exposed to 0.09 mSv of total body radiation from HR-pQCT.

E.11.c.iv. Total Radiation Exposure for participants at CUIMC: Total body radiation exposure delivered from spine X-rays, 3 DXA scans, and 3 HR-pQCT scans is 0.72mSv. Compared total body radiation due to a year's worth of background radiation (3.1 mSv), 0.72 mSv is approximately equivalent to 85 days of radiation.

E.11.c.v. Total Radiation Exposure for participants at non-CUIMC sites: Total body radiation exposure delivered from spine X-rays and 3 DXA scans is 0.63 mSv. Compared total body radiation due to a year's worth of background radiation (3.1 mSv), 0.63 mSv is approximately equivalent to 74 days of radiation.

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