Denosumab to Prevent High-Turnover Bone Loss After Bariatric Surgery

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Version 2.3

VERSION TRACKING LOG (reverse chronological order)

File Name	Version Date and Number	Version Notes	Summary of changes
2022-12-13_Detailed_Protocol	2022-12-13 V2.3	Amendment	• Added 24-hour urine collection to 19-month labs
2022-06-10_DetailedProtocol	2022-06-10 V2.2	Amendment	• Moved 2-month, 8-month, and 14-month visits from Phase 1 (intensive protocol) to Phase 2 (standard protocol) • Removed serum banking at 2-month, 8-month, and 14-month visits for Phase 2 • Removed 6-week phone call from Phase 1 • Adjusted compensation to $850 for all participants
2022-04-04_DetailedProtocol	2022-04-04 V2.1	Amendment	• Added an AE assessment 3-5 days after the zoledronic acid infusion
2022-02-17_DetailedProtocol	2022-02-17 V2.0	Amendment	• Added Phos to 19-month labs • Moved 24-hour urine collection at Randomization, 7-month, and 13-month from intensive protocol to standard protocol
2021-03-26_DetailedProtocol	2021-03-26 V1.9	Amendment	• Changed eGFR requirement for injections #s 2 and 3 to ≥30, provided no hypocalcemia • Added optional retesting of SV2 25OHD level
2020-12-31_DetailedProtocol	2020-12-31 V1.8	Amendment	• Made weight limit exclusion study site specific • Added HIV-related exclusion criteria • Adjusted/clarified TSH and hyperthyroidism exclusion criteria
2020-11-24_DetailedProtocol	2020-11-24 V1.7	Amendment	• Added optional retesting of SV1 screening labs (25OHD,eGFR,PTH) • Added hypophosphatemia as potential risk • Added measurement of serum phosphorus and magnesium levels to intensive-monitoring protocol • Clarified anthropometrics including waist circumference
2020-09-18-DetailedProtocol	2020-09-18 V1.6	Amendment	• Clarified postmenopausal status definition for women 25-45

			• Modified exclusion criteria regarding liver disease, estradiol use, dental implant/extraction • Added exclusion criterion: solid organ transplant • Expanded window for study drug visit at 7mo, 13mo, & 19mo to allow 2 weeks prior and 4 weeks after expected due date • At SFVAHCS/UCSF, consent may be obtained virtually • Clarified that participants will not be randomized without high-quality baseline DXA • Ensure Vit D supplementation of at least 3000IU/day • Added action if 25OHD >100 ng/mL
2020-08-16-DetailedProtocol	2020-08-16 V1.5	Amendment	• Added recruitment from regional bariatric surgery clinics
2020-06-22-DetailedProtocol	2020-06-22 V1.4	Amendment	• Allow option for screening consent to be verbal. Eliminated specification of "written" informed consent for the screening consent form.
2020-01-31-DetailedProtocol	2020-01-31 V1.3	Response to VA re: CRADA, response to DSMB	• Clarified SF site's institution • Modified exclusion criterion regarding prior oral bisphosphonate use to require a wash-out period • Revised and clarified Safety Review Plan and Monitoring: when 25OHD level is 20-24 ng/mL; severe osteoporosis; general considerations
2019-11-27_DetailedProtocol	2019-11-27 V1.2	Resubmission to MGH and UCSF IRBs, response to FDA	• Corrected NCT# on page 7 • Updated to reflect that IND has been obtained • Added more details of randomization and study drug administration • Moved zoledronic acid to main protocol rather than extension study • Added premedication with acetaminophen for zoledronic acid infusion • Added exclusion criteria
2019-10-09_DetailedProtocol	10/9/19 V1.1	Initial UCSF IRB and VA R&D submission	• Completed the final sentence of section VI. Study Protocol → Safety Measures • Adjusted formatting of Version Tracking Log

2019-09-22_DetailedProtocol	V1.0	Initial MGH IRB submission	• Added version tracking log and #, removed version date • Added IND # • Added NCT # • Added QCT hip at months 1 and 19 for all subjects; will offset scanning to focus on left hip and minimize beam hardening artifact • Removed radiation exposure estimates, as these will be provided by Radiation Safety Committee in Consent form
2019-09-13_DetailedProtocol CLEAN	9/13/19 (unnumbered)	n/a sent to FDA as part of IND submission	• Removed tracked changes from prior
2019-09-10_DetailedProtocol tracked[EY]	9/10/19 (unnumbered)	Approved by Amgen	• Added ZOL extension study using tracked changes

I. BACKGROUND AND SIGNIFICANCE

Severe obesity (BMI ≥40 kg/m^2) affects one in thirteen US adults, and rates continue to rise.[1] Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) have emerged as the most popular and effective treatments for severe obesity, encompassing 90% of all bariatric surgeries performed in the US.[2,3] However, these bariatric procedures have the unintended consequence of inducing metabolic bone disease.[4,5] We previously documented bone mineral density (BMD) declines of ~10% within the first 2 years after RYGB surgery despite weight stabilization.[6,7] Bone loss is accompanied by deleterious changes in cortical and trabecular bone microarchitecture[8,9] that contribute to a 13-20% decrease in estimated bone strength.[10,11] Postmenopausal women appear to be particularly at risk for enhanced bone loss.[6,12] We and others have demonstrated that SG also results in substantial declines in bone density, potentially equivalent in magnitude to RYGB.[13,14] These skeletal defects can occur despite aggressive calcium and vitamin D supplementation and without secondary hyperparathyroidism. Notably, bariatric surgery increases fracture risk at clinically relevant sites, including the hip.[15-18] As the bariatric surgery population increases further in number and age, it is critical to develop strategies to reduce this increased fracture risk.

We have shown that dramatically increased bone resorption is the primary mechanism by which bariatric surgery induces bone loss. By 6 months after RYGB, serum CTX peaks at 275% above baseline levels,[19] then it persists above baseline for at least 5 years after surgery.[10,20] Dramatic increases in CTX also occur after SG, peaking within the first postoperative year.[14] Importantly, bone loss at axial sites appears to be greatest in the first postoperative year.[12,20] Thus, it stands to reason that treatments that block early bone resorption might prevent the clinically significant bone loss induced by bariatric surgery.

Early studies in this field relied solely on dual-energy x-ray absorptiometry (DXA) for assessment of BMD, but DXA may be subject to artifactual inaccuracies in obesity and during weight loss.[21] Recent studies have used advanced non-invasive skeletal imaging technologies such as quantitative computed tomography (QCT) and high-resolution peripheral QCT (HR-pQCT) to circumvent many of these difficulties, and also to gain insight about structural and microarchitectural properties of bone.[6,11] Additional tools, including evaluation of cortical porosity and assessments of bone strength with micro-finite element analysis, have further improved characterization of skeletal fragility after bariatric surgery. These cutting-edge tools can provide a comprehensive assessment of skeletal strength after bariatric surgery.

Denosumab (DMAB) is a highly potent antiresorptive agent that has been shown to improve bone density and reduce fractures in postmenopausal women with osteoporosis[22] and to improve bone density in men with osteoporosis.[23] However, the ability of DMAB to prevent high-turnover bone loss after bariatric surgery has not been studied.

II. SPECIFIC AIMS

Our overall objective is to inhibit high bone turnover, prevent bone loss, and ultimately reduce fracture risk in at-risk adults undergoing either RYGB or SG. This protocol is a 2-site

randomized controlled trial in 36 postmenopausal women and men ≥50 years old undergoing RYGB or SG, randomized 2:1 to DMAB or placebo, with the following aims:

Aim 1. Evaluate the effect of denosumab treatment on BMD and bone turnover in postmenopausal women and older men undergoing RYGB or SG. We will examine the effect of 18 months of DMAB versus placebo, administered beginning 1 month postoperatively, on total hip BMD change by DXA. Secondary outcomes will include changes in spine BMD by DXA, spine and femoral BMD by QCT, and in bone turnover markers CTX and P1NP.

- Hypothesis 1a: DMAB attenuates early bariatric surgery-induced bone loss.
- Hypothesis 1b: DMAB inhibits the increases in CTX and P1NP after bariatric surgery.

Aim 2. Determine the effect of DMAB treatment on serum calcium level in postmenopausal women and older men undergoing RYGB or SG. In an initial subgroup of participants, we will perform intensive monitoring of serum calcium levels, confirming the key time points for targeting monitoring for subsequent participants. For all participants, we will employ a carefully designed peri- and postoperative protocol of calcium and vitamin D supplementation.

- Hypothesis: With appropriate supplementation and monitoring, DMAB lowers serum calcium but does not lead to higher rates of postoperative hypocalcemia than placebo.

Aim 3. Assess changes in bone microarchitecture and estimated bone strength after DMAB in postmenopausal women and older men undergoing RYGB or SG. On an exploratory basis, we will utilize HR-pQCT to assess skeletal changes after bariatric surgery.

- Hypothesis: DMAB prevents deterioration of bone microarchitecture and estimated bone strength.

On an ***exploratory basis***, we will examine the effect of open-label zoledronic acid on hip and spine BMD by DXA in participants from both DMAB and placebo arms of the trial. A single infusion of zoledronic acid will be administered 6 months after the last denosumab vs. placebo injection. We hypothesize that zoledronic acid maintains BMD in those treated with DMAB in the immediate postoperative period, and increases BMD in those untreated in the immediate postoperative period.

III. STUDY OVERVIEW

We will conduct a randomized placebo-controlled trial of the efficacy and safety of DMAB to prevent high-turnover bone loss in postmenopausal women and men ≥50 years old undergoing RYGB or SG. Thirty-six participants will be assigned to DMAB or placebo for 18 months. This protocol involves 2 phases: Phase 1 will involve intensive monitoring of laboratory values and symptoms to examine safety of denosumab in a bariatric surgery population and to more closely characterize changes in biochemical markers of bone turnover. Phase 2 will be a standard protocol which still monitors for safety but requires fewer study visits. The Phase 1 protocol will remain in effect until ALL of the following criteria are met:

(1) At least 12 study subjects overall finish the postoperative 7-month study visit;
(2) At least 3 RYGB study subjects at each site finish the postoperative 7-month visit;
(3) At least 8 study subjects overall finish the postoperative 13-month study visit;
(4) The Data Safety Monitoring Board (DSMB) approves the completion of Phase 1.

Once these criteria are met, all remaining study subjects (including those actively on protocol) will move to the Phase 2 standard protocol.

At the conclusion of the 18 months of DMAB or placebo, study subjects in both treatment arms will receive a single infusion of open-label zoledronic acid. Final study assessments will be performed 24 months postoperatively.

This 2-site protocol is an investigator-initiated study that is being funded through the Investigator Sponsored Studies program at Amgen Inc. Each site will receive independent approval from their local IRBs (MGH/Partners Human Research Committee and UCSF/SFVAHCS).

IV. SUBJECT SELECTION

Inclusion criteria:

1. Women or men who are planning RYGB or SG surgery
2. Women must be aged ≥ 25 years AND postmenopausal by one of the following criteria:
   > 36 months since last spontaneous menses; for women aged 25-45, documentation required that there are no secondary causes of amenorrhea plus serum FSH > 40 units/liter; or
   >36 months since hysterectomy plus serum FSH > 40 units/liter, or clear record of > 36 months since bilateral oophorectomy in the medical chart
3. Men must be aged ≥ 50 years

Exclusion criteria:

1. Prior bariatric surgery
2. Weight ≥ study site limit (determined by weight limit for bone imaging equipment)
3. Renal disease (estimated GFR < 60 mL/min/1.73 m^2 at Screening Visit 1 (SV1)* or at Screening Visit 2 (SV2))
4. Hypercalcemia (albumin-adjusted Ca > 10.5 mg/dL) or hypocalcemia (albumin-adjusted Ca < 8.5 mg/dL) at SV1* or at SV2
5. Hypomagnesemia (Mg < 1.7 mg/dL) at SV1* or at SV2
6. Serum 25-OH vitamin D (25OHD) < 20 ng/mL at (or if assessed on clinical laboratory testing, within 3 months before) SV1,* or 25OHD < 25 ng/mL at SV2
7. Hyperparathyroidism (PTH > upper normal limit) at (or if assessed on clinical laboratory testing, within 3 months before) SV1*
8. Liver disease with AST or ALT >2 times upper limit of normal at SV1**, or history of cirrhosis with INR ≥ 1.3 or T.bili ≥ 1.3 mg/dL or history of decompensation
9. HCT < 32% at SV1**
10. History of malignancy (except basal cell carcinoma) in the past 1 year
11. Significant cardiopulmonary disease (unstable coronary disease or stage D ACC/AHA heart failure)
12. Major psychiatric disease
13. History of celiac disease or inflammatory bowel disease
14. History of solid organ transplant
15. Excessive alcohol or substance abuse

16. Paget's disease, primary hyperparathyroidism, or any other known congenital or acquired bone disease other than osteoporosis
17. TSH <0.3 (with TSH to be checked at SV1* if potential participant reports any history of hyperthyroidism or takes thyroid hormone supplement)
18. History of HIV infection with current CD4 count ≤ 500 cells/mm3 measured at SV1*, or history of AIDS-defining illness
19. Current use of loop diuretics
20. Current use or use in the past 12 months of DMAB
21. Current use of oral bisphosphonates, or prior use if last use is more recent (in years) as the number of years of cumulative oral bisphosphonate exposure (i.e., Last use must be at least as many years ago as the cumulative oral bisphosphonate use. Maximum required washout is 5 years, so if cumulative oral bisphosphonate exposure is >5 years, last use must be at least 5 years ago.)
22. Current use or use within the past 3 months of SERMs or calcitonin
23. Current use or use within the past 3 months of systemic (oral, transdermal) estrogen
24. Use of testosterone therapy if dose has changed within the last 3 months, or if dose change or discontinuation is planned in the upcoming 18 months
25. Any current or previous use of teriparatide, strontium, or any parenteral bisphosphonate
26. Use of oral or parenteral glucocorticoids for more than 14 days within the past 6 months
27. Current use of anti-VEGF drug
28. Suspicion for unhealed dental extraction/implant at time of randomization visit, <4 weeks between last dental extraction/implant and start of study medication, or future planned extensive dental work involving extraction or dental implant in the 24 months after Randomization Visit
29. DXA BMD T-score of < -3.0 at PA spine, total hip, or femoral neck

* New measurement at SV1 not needed if the analyte was measured on clinically-obtained laboratory testing within 3 months before SV1.
** New measurement at SV1 not needed if the analyte was measured on clinically-obtained laboratory testing within 12 months before SV1.

Recruitment:
At the Massachusetts General Hospital (MGH) and San Francisco Veterans Affairs Health Care System (SFVAHCS, academic affiliate University of California, San Francisco, UCSF) sites, study subjects will be recruited from the bariatric surgery clinics (the MGH Weight Center, the UCSF Bariatric Surgery Center, the SFVAHCS Obesity Surgery Clinic, and regional bariatric surgery clinics). Briefly, we will advertise the study using IRB-approved materials within the medical centers and clinics. Recruitment flyers will be posted in clinic and by website, and email and online announcements will be distributed. A potential subject may be identified by his or her treating physician at the clinic and through the surgical scheduling calendar. The subject will be sent a letter (via mail, email and at UCSF via the secure patient messaging portal) to introduce the study. Letters sent by mail will be co-signed by the treating physician and the study PI. A member of the study staff who is not the treating physician will also explain the study to each potential bariatric surgical subject at a pre-operative visit or over telephone or video-conference after the pre-operative visit. With permission from the treating physician, we may access a potential subject's medical record to confirm contact information and to identify upcoming visits

at the Weight Center. A potential subject will be given a copy of the full consent form to review. He or she also will be asked permission to be contacted by study staff either during a clinical visit or by email, phone or video-conference (San Francisco site only) to further discuss the study.

V. SUBJECT ENROLLMENT

The study will enroll a total of 36 subjects between both sites who are planning RYGB or SG surgery. We anticipate that 18 subjects will be recruited at MGH and 18 at SFVAHCS/UCSF. Subjects will be randomized to receive DMAB or placebo in a 2:1 ratio (i.e. 24 DMAB and 12 placebo). We will enroll men age ≥ 50 and postmenopausal women.

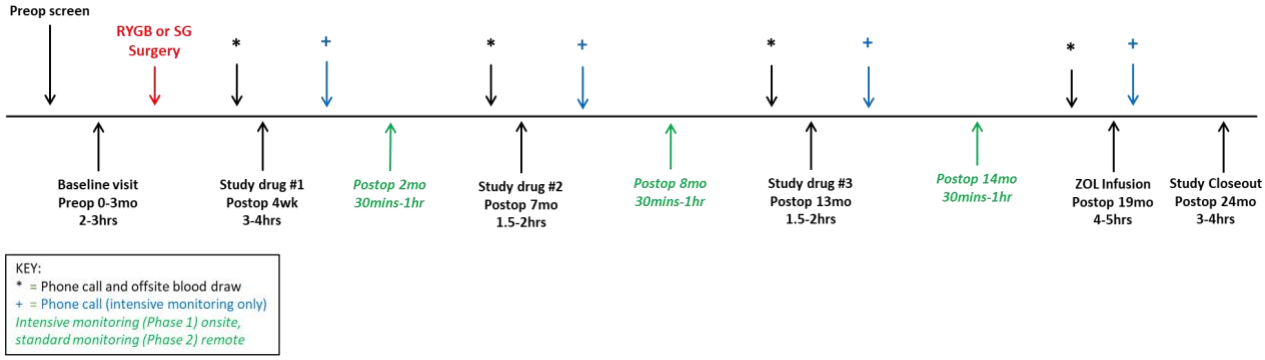
To ensure compliance with HIPAA regulations, subjects that will be pre-screened by telephone are informed of the nature and sensitivity of all questions at the beginning of the conversation. They will be asked whether this is an appropriate time for them to answer these questions and told how long the phone call is expected to take.

Subjects will be asked questions about their medical history to determine their eligibility for the study. If they are found to be eligible based on the screening questionnaire, informed consent for the pre-operative screening procedures will be obtained by a licensed physician, nurse practitioner, or study coordinator at Screening Visit 1 (SV1). In situations where a nurse practitioner or study coordinator is obtaining consent, subjects will be offered the option for further discussion with a physician investigator if they have any remaining questions. This offer will be documented in the research record, and any consent problems will be reported to the local IRB in real-time. Subjects will be provided with a copy of the signed screening consent form, as well as a copy of the unsigned, full study consent form for review. The pre-op screening procedure involves a blood draw to assess whether subjects meet all remaining eligibility criteria. If labs and medical history show that SV1 eligibility criteria are met, the individual will be invited to a Baseline Visit, at which informed consent for the main study will be obtained. At SFVAHCS/UCSF, informed consent for the main study may also be obtained in advance of the Baseline Visit by video conferencing.

Written informed consent for the full study will be obtained using a consent form before subjects undergo any study procedures other than the screening procedure described above. All subjects will be informed regarding the purpose of the research, the details of the study protocol, risks and benefits, alternatives to participation, costs, reimbursements, their right to privacy and confidentiality, their right to refuse to participate or withdraw from the study at any time, their rights in the event of a study-induced injury, and whom to contact for questions about the study. A complete medical history will also be taken to ensure the subject is an eligible candidate for the study. Hospital interpreters will be available for subjects for whom English is not their primary language, and local IRB guidelines will be followed regarding whether these subjects will sign an additional short-form as well as the primary consent form. Subjects will be given a copy of all signed consent forms and an additional copy of each consent form will be kept in our research files.

Subjects will receive a total $850 compensation for participating in all visits of the intensive-monitoring protocol, or for participating in all visits of the standard protocol. In addition, subjects will receive a parking voucher or transportation compensation for each visit. (Local limits to transportation compensation may apply.)

VI. STUDY PROTOCOL



The study schema for the main trial is shown above. A detailed table of study measurements (Appendix A) describes the visits and procedures to be performed (see separate file). The study PI (Elaine Yu, MD) has obtained an IND (#145467) from the FDA, and the trial is registered on clinicaltrials.gov (NCT #04087096).

Screening and baseline assessments

Study subjects will be identified prior to surgery and will undergo initial screening procedures at Screening Visit 1 (SV1) to determine initial eligibility for the study. If a participant is found to be eligible based on the inclusion and exclusion criteria above, he or she will be scheduled for a Baseline Visit, to be performed no more than 3 months before the scheduled operation. If a participant does not meet eligibility criteria based on 25OHD, eGFR, and/or PTH criteria, and is otherwise eligible to participate, then a study nurse or physician may make recommendations to the participant about vitamin D supplementation and/or hydration and retest the disqualifying labs.

The Baseline Visit will involve laboratory testing (Ca, Phos, Alb, Cr, and 25OHD) and serum banking for future studies (e.g. bone turnover markers), DXA for BMD and body composition, anthropometric measurements (height, weight, waist circumference and elbow breadth to estimate frame size), vitamin D and calcium intake screening, and a physical activity questionnaire. Subjects will be provided individualized calcium and vitamin D supplements to start immediately, and ergocalciferol 50,000 IU to take daily for 3 days prior to the second screening visit and ideally before surgery.

After surgery, subjects will undergo post-op screening procedures at Screening Visit 2 (SV2) between 1.5 and 4 weeks postoperatively. The SV2 involves repeat laboratory testing (Ca, Alb,

Cr, and 25OHD), the vitamin D and calcium intake screeners, and a post-op interview to confirm patient tolerance of oral supplements and assessment of any perioperative complications. The SV2 visit can occur on site, or with blood drawn at a local Quest patient services center or other clinical laboratory (e.g., the SFVAHCS clinical lab) with the interview and calcium/vitamin D intake assessment performed over the phone. If a participant does not meet SV2 eligibility criteria based on 25OHD level, and is otherwise eligible to participate, then a study nurse or physician may make recommendations to the participant about continued or increased vitamin D supplementation and retest the 25OHD level.

If all eligibility criteria are met at SV2, a subject is scheduled for a Randomization Visit, which will occur within 3-6 weeks after surgery. If 25OHD level is 25-29 ng/mL at SV2, then at least one week will be ensured between SV2 and the Randomization Visit in order to allow time for additional vitamin D therapy, as described in the Safety and Monitoring Plan below. The Randomization Visit will involve additional skeletal imaging (QCT, HR-pQCT), laboratory testing, serum banking, the calcium/vitamin D intake screener, and then randomization to receive either DMAB or placebo. (See Randomization Procedures, below.)

Subsequent visits can be split into three types: visits that must be done onsite (displayed in black in the study schema); visits that may be done on site or remotely by phone and/or at a local Quest patient services center or other clinical laboratory (indicated by * or + in the study schema); or visits that are specific to the intensive monitoring protocol (blue in the study schema). Intensive monitoring visits will only occur during Phase 1. All other visits will occur as part of the standard protocol during Phases 1 and 2. If participants begin the study while it is being conducted with the intensive protocol, and their later study visits transition to use the standard protocol instead, they will be notified of this change via phone or email during one of their usual study communications.

Standard protocol

Following the Randomization Visit, subsequent onsite visits will occur every 6 months, at postoperative months 7, 13, and 19 (- 2 weeks, +4weeks). The standard protocol onsite visits involve imaging (DXA at 7, 13, and 19 months post-op; QCT at 19 months post-op; HR-pQCT at 19 months post-op); laboratory testing (Ca, Alb, Cr, 25OHD, and Phos at the 19 month post-op); serum banking for subsequent analysis of PTH and bone turnover marker levels; 24-hour urine collections for urinary Ca and Cr at 1, 7, 13, and 19 months post-op; adverse event (AE) assessment; and study drug injection (DMAB or PBO at 7 and 13 months post-op).

Before each of the remaining onsite visits, participants will have laboratory testing (including Ca, Cr, and 25OHD) and assessment of calcium and vitamin D intake (green visits in the study schema). These lab tests can occur on site, or remotely by phone and with lab testing performed at a local commercial lab patient services center or other clinical laboratory.

Following the 1, 7, and 13 month post-op visits participants will have laboratory testing (including Ca, Phos, Mag, Alb, Cr, and 25OHD as needed) and assessment of calcium and vitamin D intake (green visits in the study schema). These lab tests can occur on site, or remotely

by phone and with lab testing performed at a local commercial lab patient services center or other clinical laboratory.
At the 19 mo post-op visit, study subjects in both treatment arms will receive a single infusion of open-label zoledronic acid. Three to five days after the zoledronic acid infusion (19 mo post-op visit), subjects will receive a phone call to assess for AEs and to confirm and reinforce continued compliance with calcium and vitamin D supplements.

Participants will return for a 24 mo post-op visit for DXA imaging, laboratory testing, serum banking, and AE assessment.

Intensive-monitoring protocol

In addition to the Standard Protocol visits listed above, Phase 1 involves additional intensive monitoring as an additional safety measure.

- There will be in-person visits 1 month after each study drug administration (i.e. at 2, 8, and 14 months post-op) for lab testing (serum Ca, Phos, Mag, Albumin, Cr), serum banking, AE assessment, and calcium/vitamin D intake questionnaires. These visits will continue off-site during Phase 2, without serum banking.
- There will be phone calls 2 weeks after study drug injections #1, #2 and #3 (i.e. at 6.5, 7.5 and 13.5 months post-op) for AE assessment and to confirm continued compliance with calcium and vitamin D supplements.

Calcium and vitamin D provision and counseling

Subjects will receive personalized nutritional (calcium/vitamin D) counseling throughout the study to ensure compliance with guidelines for the postoperative management of bariatric surgery patients.[24] At the Baseline Visit and then frequently during the study (see study schema and table of study measurements), subjects will complete a validated calcium/vitamin D intake screener,[25] and based on dietary and multivitamin intake, a chewable calcium citrate tablet will be dispensed at a dose designed to achieve a total calcium intake (diet plus supplements) of at least 1500 mg/day. This will replace any stand-alone calcium supplement product, although subjects will be permitted to continue their own multivitamins even if they contain calcium. (Calcium within the multivitamin will be included in the total calcium intake.) Vitamin D intake will be assessed, and a vitamin D supplement will be dispensed if appropriate to ensure a vitamin D intake of at least 3000 IU/day. This will replace any stand-alone vitamin D product, although subjects will be permitted to continue their own multivitamins even if they contain vitamin D. Of note, most RYGB and SG patients at our institutions are prescribed by their surgeons a standardized postoperative diet plan that includes 1000-1200mg of dietary calcium for the initial 2 postoperative weeks.

During post-op follow-up, repeat administration of the calcium/vitamin D intake screener and repeat measurement of 25OHD level will result in individualized adjustments in calcium supplement dose and vitamin D supplement dose, to ensure post-operative total calcium intake of at least 1500 mg/day of calcium and 25OHD level of at least 30 ng/mL, in accordance with guidelines for the postoperative management of bariatric surgery patients.[24] Whenever 25OHD level is measured following the administration of study drug injection #1, if 25OHD level is

>100 ng/mL, then the study physician will instruct the participant in decreasing his or her vitamin D supplement dose.

Randomization procedures

Study subjects will be randomized 2:1 to either denosumab or placebo. Randomization will be stratified by site (i.e. MGH or SFVAHCS/UCSF) and procedure type (i.e. RYGB or SG). Each site's investigational pharmacy will be responsible for preparing and maintaining separate lists of randomization numbers for RYGB and SG. Each surgical group at each site will be randomized in blocks of 3 to maintain denosumab:placebo balance. Furthermore, as enrollment gets closer to goal, MGH Pharmacy and UCSF Pharmacy will contact each other to ensure that denosumab:placebo enrollment for the overall study is balanced in consultation with the site PIs. Participants will not be randomized if they do not have a high-quality DXA.

Study drug administration

Over the course of the study, subjects will receive a total of 3 injections of either denosumab 60 mg SC q6 months or placebo injection SC q6 months. Study subjects in both treatment arms will subsequently receive a single infusion of zoledronic acid 5 mg IV over 20 minutes.

Study measurements

A detailed table of study measurements and schedule is shown in Appendix A. Briefly, the following data will be collected:

1. DXA BMD measurements of the total body, lumbar spine, total hip, femoral neck, and 1/3 radius and body composition measurements
2. QCT measurements of the lumbar spine and proximal femur
3. HRpQCT measurements of the distal radius and tibia
4. Anthropometric measurements of height, weight and waist circumference
5. Fasting blood samples for labs (including Ca, Cr, 25OHD)
6. Batched serum samples for bone turnover markers CTX and P1NP, and PTH
7. Screen of dietary intake of calcium and vitamin D
8. Questionnaires
    a. Medical history
    b. Physical activity
    c. Adverse events

<u>DXA</u>
Dual-energy x-ray absorptiometry (DXA) scans for areal BMD (aBMD) of the total body, lumbar spine, total hip, femoral neck, and 1/3 radius and for body composition will be obtained at baseline and 7, 13, 19, and 24 months post-op (Hologic, Inc., Bedford, MA). The short-term precision for this technique for aBMD is 1-2% at the MGH Bone Density Center.

<u>QCT</u>
Quantitative computed tomography (QCT) scans of the lumbar spine and proximal femur will be obtained at the randomization visit and 19mo post-op (GE CT scanner, GE Healthcare,

Waukesha, WI). These scans will be used to assess volumetric bone mineral density (vBMD). Scans are performed with helical acquisition, 1.25mm slice thickness, and QCTPro calibration phantom. Analysis of the QCT images will be performed using Mindways QCT Pro software. 3D reconstructive analysis for volumetric bone density for integral, trabecular and cortical compartments will be performed on QCTPro software (Mindways Software, Inc., Austin, TX). The short-term precision for this technique is 1-2% for vertebral BMD measurements.[26,27]

HR-pQCT

High-resolution peripheral QCT (HR-pQCT) scans of the distal (4%) radius and distal tibia (7.3% and 30%) will be obtained at the randomization visit and 19mo post-op (Xtreme CT II, Scanco Medical, AG, Basserdorf Switzerland) to assess bone microarchitecture. We will scan the non-dominant radius and tibia, unless they have previously had a fracture of that arm or leg. We will assess volumetric bone density and trabecular microarchitecture using standard manufacturer software, and will use newly developed algorithms for cortical thickness and porosity. Scans will be reviewed for movement artifacts and statistical analyses performed with and without measurements with moderate/severe movement artifacts. Quality control is maintained with daily scanning of phantoms. Exams will be performed at the MGH HR-pQCT Core Facility and the UCSF Imaging Center at China Basin. Quality control is maintained with daily scanning of phantom hydroxyapatite rods embedded in a soft-tissue equivalent resin (QRM, Moehrendorf, Germany).

Anthropometric Measures

Height, weight and waist circumference measurements will be obtained at each visit without shoes using standard protocols.

Calcium and Vitamin D Intake Screener

At the Baseline Visit and then frequently during the study (see table of study measurements, Appendix A), subjects will complete a validated calcium/vitamin D intake screener.[25]

Questionnaires

Physical activity will be assessed via the International Physical Activity Questionnaire (IPAQ).[28] An additional questionnaire will assess medical history, including fracture history and medication use.

Laboratory Testing

Subjects will have real-time laboratory testing performed at the onsite visits, onsite/remote lab checks, and intensive monitoring visits, including Ca, Cr, and 25OH vitamin D using standard clinical assays. See table of study measurements (Appendix A).

Bone Turnover Markers

Fasting morning blood will be collected at each onsite visit and the intensive monitoring visits for serum banking. Serum levels of type 1 cross-linked C-telopeptide (CTX), procollagen type 1 N-terminal propeptide (P1NP) and PTH will be batched and measured at a centralized research laboratory.

Safety Measures

Following the schema, labs including Ca, alb, Phos, Cr, and 25OHD will be measured at multiple time points. Study subjects will have frequent assessment for hypocalcemic signs and symptoms and other potential adverse events. Hypocalcemia, if detected, will be graded according to common terminology for adverse event criteria (CTCAE v.4)[29] with particular attention to symptomatic hypocalcemia and/or grade 2-4 hypocalcemia (<8.0 mg/dL). Hypocalcemia will be addressed with application of an algorithm (see "Data Safety and Monitoring" section below). The first half of patients enrolled will undergo additional intensive monitoring procedures involving more frequent lab and clinical monitoring, and measurement and monitoring of 24-hour urinary calcium level.

VII. BIOSTATISTICAL ANALYSIS

Statistical methods: This randomized double-blind placebo-controlled trial is designed to demonstrate proof-of-concept that DMAB can safely prevent high turnover bone loss induced by bariatric surgery. The pilot trial will be powered to detect significant differences in total hip bone density between treatment groups (see sample size and justification below), and will provide assessments of changes in bone turnover markers and additional skeletal imaging parameters that could inform the design of future larger trials. Statistics will include descriptive as well as analytic approaches. Between-group analyses will be performed according to the intention-to-treat principle. We will attempt to collect complete follow-up data on every randomized participant, regardless of adherence to treatment. As randomization in this small sample may not result in the perfect between-group balance of relevant variables (e.g., age, sex, weight), we will perform analyses adjusting for any of these that are substantially imbalanced.

We will compare changes in total hip BMD from pre-operative baseline to post-operative month 19 (primary endpoint) by treatment group (DMAB vs. placebo) using linear mixed models with random subject intercepts, fixed time, site, and treatment main effects, and fixed time x treatment interaction to determine differences between treatment groups and over time; secondary analyses will also adjust for age, sex, weight and evaluate differences by bariatric surgery type. For secondary bone endpoints, we will evaluate between-group changes changes in spinal BMD by DXA (pre-op baseline to month 19) and quantitative computed tomography (QCT; post-op month 1 to month 19), and in the bone turnover markers CTX and P1NP (pre-op baseline to month 19) using linear mixed models. For the comparisons within specific postoperative timeframes of 7 and 13 months, the mixed modeling will incorporate linear contrasts. Additional analysis of secondary endpoints will use Fisher's exact test to compare incidence of any hypocalcemia (grade 1-4) and, separately, hypocalcemia that is grade 2-4 in the two treatment groups. In the first 12 subjects, on an exploratory basis, we will describe in detail the net effects of DMAB and bariatric surgery on biochemical markers of bone turnover and on serum and urinary calcium levels. In additional exploratory analyses, we will compare changes in bone microarchitecture and estimated strength by treatment group (post-op month 1 to month 19) using linear mixed models. Finally, data from the zoledronic acid portion of the study will be analyzed on an exploratory basis, focusing both on changes in BMD and bone turnover markers between month 19 and month 24, and on changes between pre-op baseline to month 24. Subjects with missing data will be retained in the linear mixed models to the extent that data are available. Even though the mixed model provides unbiased estimates in the presence of small incomplete

subjects with random occurrence of missingness (MAR) over the postoperative times, we will conduct a parallel analysis to incorporate multiple imputation into the mixed model analysis. To evaluate factors that may influence bone loss after bariatric surgery, we will also examine correlations of change in bone outcomes with change in physical activity, body composition, serum 25-hydroxyvitamin D levels, and serum PTH.

Sample size and power calculations: The sample size of 36 will allow detection of significant differences in total hip BMD between treatment groups, and will further allow us to estimate effects on secondary and exploratory outcomes with reasonable precision.

Primary endpoint: Based on data from our previous longitudinal RYGB and SG studies,[13] and assuming a dropout rate of 15%, a sample of 36 with 2:1 randomization will provide 80% power in 2-sided tests to detect an absolute between-group difference of 4% in total hip BMD percentage change. These are plausible effects, given that our previous observational studies have shown 1-year BMD changes of 9% in the absence of treatment. Thus, even if BMD declines by 5% in the presence of denosumab, we will have 80% power to detect a significant difference between groups. If we denosumab instead prevents all but 2% of bariatric surgery induced bone loss, our power will increase to 99%.

Secondary BMD and bone turnover endpoints: Based on our prior studies, this sample size will also provide 80% power to detect between-group differences of 6% for spine BMD by DXA, 7% for femoral neck BMD by DXA, and 5% for trabecular spine BMD by QCT. In addition, we will be able to detect a difference of 102% between groups in CTX percentage change. Note that our previous observational studies have shown CTX increases of 200-278% in the absence of treatment within the first year of RYGB.[6,7]

Secondary calcium endpoint: We recognize that power will be limited in this pilot trial: for example, if just one (10%) placebo subject develops hypocalcemia, we will only be able detect a between-group difference of 54%. Nonetheless, results will be useful for designing a subsequent larger trial. Furthermore, we plan to intensively phenotype the calcium kinetics within the first 12 patients enrolled in this study. We expect to see no symptomatic grade 2 or any grade 3-4 hypocalcemia and would consider such a result reassuring to continue enrollment with a lower intensity monitoring protocol for the completion of the trial. Likewise, we would be reassured if only a few subjects develop mild hypocalcemia and it responds easily to our carefully designed algorithm.

Exploratory endpoints: Based on SDs from our previous longitudinal studies,[11] this sample size will provide 80% power to detect between-group differences of 5-6% in total vBMD and 6-7% in estimated failure load at the radius and tibia by HR-pQCT.

VIII. RISKS AND DISCOMFORTS

Denosumab

Denosumab (DMAB) is an FDA approved medication used to treat osteoporosis in various populations, including postmenopausal women. More than 13,500 patients have been treated

with DMAB in clinical studies, and it is generally well tolerated. In clinical studies, it has been reported that DMAB may produce the following side-effects: eczema, serious skin infections, low blood calcium (which can cause tingling in the fingers or around the mouth, muscle cramps, or abnormal heart rate), pain in the joints or extremities, high blood cholesterol, dizziness, cough, difficulty emptying the bladder, and decreased skin sensation. Hypocalcemia has been rarely reported in absence of renal failure; see below for additional discussion. Osteonecrosis of the jaw has been reported rarely in women with postmenopausal osteoporosis and in the oncology clinical trial program in patients treated with DMAB. To reduce the likelihood of adverse events, subjects with any contraindications to DMAB, including dental procedures as outlined in the eligibility criteria, will be excluded. Subjects will be monitored for report of thigh or groin pain and would be evaluated to rule out an atypical femur fracture. There is concern for an increased risk of fracture when DMAB is discontinued and not followed by subsequent osteoporosis treatment. All study subjects will receive an infusion of zoledronic acid following the denosumab or placebo intervention, and subjects who withdraw consent during the study and prior to zoledronic acid will be counseled again at the time of withdrawal about this risk.

Hypocalcemia and other mineral abnormalities

It is possible that RYGB patients may be more susceptible to developing hypocalcemia in response to DMAB due to known reductions in calcium absorption that have been documented to occur after surgery.[19,30] The incidence of postoperative hypocalcemia (<8.9 mg/dl) after RYGB is 1-2% and is associated with pre-existing renal insufficiency and vitamin D deficiency.[31,32] Study subjects in this trial will be excluded for renal insufficiency (eGFR <60 mL/min/1.73 m^2) and vitamin D deficiency, as well as for low serum calcium or magnesium, or use of loop diuretics. Low magnesium can potentiate the risk of hypocalcemia after DMAB, and DMAB administration also carries the potential risk of hypophosphatemia. For safety monitoring, in the intensive-monitoring protocol, serum phosphate and magnesium levels will be measured following study drug administration (in addition to calcium and creatinine as outlined in the standard-monitoring protocol). All study subjects will be provided individualized calcium citrate and vitamin D supplements throughout the trial, ergocalciferol 50,000 IU for 3 days prior to first study drug administration and if needed in response to decreases in 25OHD level, and personalized counseling about calcium and vitamin D intake and adherence throughout the study. We will monitor serum calcium, 25OHD, and renal function. ***See X: Data and Safety Monitoring Plan – Safety Review Plan and Monitoring (below) for the detailed measures to be taken to decrease risk of hypocalcemia.***

Ergocalciferol

There is a very small risk that the ergocalciferol supplement could cause vitamin D toxicity. This is unlikely to be an issue for the dose used in this study.

Calcium and Vitamin D Supplements

The MGH Weight Center, UCSF Bariatric Surgery Center, and SFVAMC Obesity Surgery Clinic recommends that all RYGB and SG patients take supplemental calcium and vitamin D. These supplements will be provided to study subjects during their participation in the trial. In the doses used in this study, calcium and vitamin D have minimal side effects. Some individuals may note constipation or stomach upset with calcium supplements. Very rarely, if an individual is over-supplemented with calcium, a kidney stone could result.

Zoledronic Acid
The most common side effects associated with zoledronic acid include flu-like symptoms (e.g., fever, chills, muscle/joint aches) occurring after the infusion. The majority of these symptoms occur within the first 3 days following drug administration and usually resolve within 3 days of onset but resolution can take up to 7-14 days. Taking acetaminophen or ibuprofen after the infusion can mitigate these symptoms, and acetaminophen will be administered to trial participants unless contraindicated. Other common side effects include nausea, tiredness, dizziness, headache, or pain/redness/swelling at the injection site.

Hypocalcemia
Zoledronic acid, like all potent antiresorptive agents, has also been associated with rare cases of hypocalcemia. In the pivotal clinical trial of zoledronic acid for postmenopausal osteoporosis (n=7736), approximately 0.2% of patients had notable declines of serum calcium levels to values less than 7.5 mg/dL within 11 days after zoledronic acid administration.[33] No symptomatic cases of hypocalcemia were observed. As noted previously, higher risk study subjects will be excluded from participation in the study, all subjects will receive personalized counseling about calcium and vitamin D intake, and we will monitor serum calcium and 25OHD. ***See X: Data and Safety Monitoring Plan – Safety Review Plan and Monitoring (below) for the detailed measures to be taken to decrease risk of hypocalcemia.***

Acetaminophen
A single dose of acetaminophen will be administered at the time of the zoledronic acid infusion in order to minimize the risk of zoledronic acid-induced flu-like symptoms. At the dose used in this trial, and with one-time use as in this trial, the risk of side effects is exceedingly low. The most common side effects are nausea, vomiting, and headache. Very rarely, acetaminophen has been associated with cases of acute liver failure, but most cases are with doses exceeding the recommended maximum daily limits. Acetaminophen will not be given if a participant has known significant liver disease or reports a sensitivity to acetaminophen.

Imaging Studies
Over the entire study period, subjects will receive 4 DXA scans of the total body, lumbar spine, hip, and 1/3 radius, 2 QCT scans of the spine and hip, and 2 sets of HRpQCT scans at the radius and 2 sites at the tibia. Scans will be performed at the timepoints outlined in the study schema above.

Blood Draws
There is some discomfort associated with blood draws and injections. Furthermore, there is a slight risk of bleeding, bruising or infections at the needle site.

Confidentiality
Participation in research may lead to unintentional loss of privacy. Although the data will be coded, stored specimens will be de-identified, and publications will not identify subjects in any way, there is nevertheless a remote possibility of loss of confidentiality with respect to stored data. To minimize this risk, all data will be kept secure and confidential in locked cabinets in locked rooms on locked wards, and in password-protected electronic files.

IX. POTENTIAL BENEFITS

Subjects may experience increases in bone density and improvements in bone microarchitecture over the course of the study. The information from the study may help in the understanding of the role of antiresorptive therapy in preventing the deterioration of skeletal health that occurs after RYGB and SG. This knowledge has the potential to mitigate one of the largest negative consequences of these surgeries, which are otherwise the most effective treatments for severe obesity.

X. DATA AND SAFETY MONITORING PLAN

Recruitment, Informed Consent and Privacy
We will enroll subjects using methods approved by the central IRB and our local IRBs and similar to methods that we have used in prior studies. The protocol and informed consent forms will be approved by our hospital's Institutional Review Board (IRB) prior to recruitment of any study subjects. Written informed consent will be obtained using an IRB-approved consent form. All subjects will be informed regarding the purpose of the research, the details of the study protocol, risks and benefits, alternatives to participation, costs, reimbursements, their right to privacy and confidentiality, their right to refuse to participate or withdraw from the study at any time, their rights in the event of a study-induced injury, and whom to contact for questions about the study. We will provide ample time for participant decision-making and an unhurried informed consent process, and we will answer any questions regarding the study or the consent. All studies will be done on mentally competent adults, only after informed consent has been obtained by the investigator and documented in writing. Subjects will be given a copy of their signed consent form and an additional copy will be kept in our research files. All protocols, consent forms, and study progress reports are reviewed annually by the IRBs and by VA regulatory personnel.

Confidentiality
During this study, medical history, physical examination and laboratory testing will be performed at baseline and at regular intervals. All of the materials collected are for research purposes only, and data will be kept in strict confidence. No information will be given to anyone without permission from the subject. The consent form includes the informed consent statements required by the MGH or SFVAHCS/UCSF. Confidentiality will be ensured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with a randomly generated identification code unique to the subject.

Database Protection
The study database will be secured with password protection and only IRB-approved study staff will have access. The study biostatistician will receive only de-identified coded information. Electronic or written communication with collaborators outside of the 2 study site teams (the PIs' teams) will involve only unidentifiable information. Adverse event reports and annual continuing reviews will not include subject- or group-identifiable material. Data will be entered into REDCap, a free, secure, web-based application designed to support data capture for research studies. REDCap was developed by a multi-institutional consortium initiated at Vanderbilt

University. Data collection will be customized for this study by the research team with guidance from Harvard Catalyst EDC Support Staff. REDCap is designed to comply with HIPAA regulations.

Safety Review Plan and Monitoring

At each of the 2 sites, the PI or other study physician will review all data collection on a weekly basis to ensure completeness, accuracy and protocol compliance. Routine safety laboratory testing will be reviewed within 48 hours of their reporting. Any abnormal laboratory testing will be reviewed in real-time. Review of the rate of subject accrual and compliance with inclusion/exclusion criteria will occur weekly during the recruitment phase and then monthly to ensure that a sufficient number of participants are being enrolled and that they meet eligibility criteria and the targeted ethnic diversity goals outlined in the grant proposal. Assessment of adverse events and unanticipated problems is described below. All participating facilities (MGH and UCSF core labs, MGH and UCSF research pharmacies) are regularly monitored as part of their participation in the Harvard and UCSF Clinical and Translational Science Award centers.

Hypocalcemia

Although rates of hypocalcemia are rare in osteoporosis studies, RYGB and SG patients might be at greater risk of developing hypocalcemia in response to DMAB. Nevertheless, we expect to show that with appropriate supplementation and monitoring, DMAB does not lead to higher rates of postoperative hypocalcemia than placebo. Phase 1, the intensive-monitoring protocol, is expected to demonstrate this in detail, such that the trial can proceed with Phase 2 (standard protocol). Based on what is learned in Phase 1, the study investigators and Data Safety Monitoring Board (DSMB, described below) may make changes to the Phase 2 standard protocol to include additional measurement(s) or procedure(s).

In all participants, to decrease risk of hypocalcemia, the following measures will be taken:

- Eligibility criteria (described above) are designed to require study subjects to have normal renal function. Potential subjects will be ineligible if they have preoperative secondary hyperparathyroidism, or if they have hypomagnesemia.
- Vitamin D deficiency will be excluded. Potential subjects will be ineligible if they have 25OHD levels <20 ng/mL at SV1. Following SV1, subjects will take ergocalciferol 50,000 IU daily for 3 days and will begin taking a calcium citrate supplement and a vitamin D supplement, according to protocol, provided by the study. If 25OHD level is <25 ng/mL at SV2, 2 weeks post-op, a subject will be excluded.
- Patients taking loop diuretics will be excluded due to theoretical increased risk of hypocalcemia.

In addition to the precautions being taken with all study subjects, postoperative serum chemistries will be monitored closely at multiple timepoints as described in the table of study measurements. Laboratory results obtained during the follow-up visits will be approached according to the following algorithm:

- Serum 25OHD < 30 ng/mL at a follow-up measurement with corrected serum calcium within normal range:

- Serum 25OHD 25-30 ng/dL: increase vitamin D intake by 2000 IU
- Serum 25OHD 20-24 ng/dL: increase vitamin D intake by 2000 IU and add ergocalciferol 50,000 IU daily for 7 days. Study drug injection should be performed only after confirmation that ergocalciferol course is completed.
- Serum 25OHD < 20 ng/dL: increase vitamin D intake by 2000 IU daily and add ergocalciferol 50,000 IU daily for 7 days. Recheck 25OHD in 2 weeks and delay next study drug injection until 25OHD at least 25 ng/mL

- Hypocalcemia:
  - Grade 1, corrected calcium 8.0-8.4 mg/dL:
    - Delay next study drug injection until corrected calcium at least 8.5 mg/dL
    - if without symptoms and if vitamin D is to be increased in response to 25OHD level (see above), no additional treatment beyond the increase in vitamin D supplement; repeat serum calcium in 1 week and if still ≤ 8.4 mg/dL then increase total daily calcium intake by 500 mg (up to a maximum daily intake of 2500 mg/day)
    - if symptomatic or if 25OHD level already at goal of 30 ng/mL, increase total daily calcium intake by 500 mg (up to a maximum daily intake of 2500 mg/day); repeat serum calcium in 1 week
  - Grade 2, corrected calcium 7.0-7.9 mg/dL:
    - Delay next study drug injection until corrected calcium at least 8.5 mg/dL
    - if without symptoms, increase total daily calcium intake by 500 mg (up to a maximum daily intake of 2500 mg/day) and if needed, replete vitamin D as per 25OHD algorithm; repeat serum calcium in 1 week
    - if symptomatic OR asymptomatic but already at maximum daily calcium intake, also add calcitriol 0.25 mcg once daily (if 7.5-7.9 mg/dL) or twice daily (if 7.0-7.4 mg/dL); repeat serum calcium in 1 week
      - If corrected calcium still <8.0 mg/dL at 1-week recheck, increase calcitriol by 0.25 mcg daily and repeat
  - Grade 3 (corrected calcium 6.0-6.9 mg/dL) or grade 4 (corrected calcium <6.0 mg/dl or severe symptoms): refer to physician for IV calcium and other evaluation and treatment. Do not give next study drug injection. This will be considered a serious adverse event and the IRB and DSMB will be notified as per reporting requirements below.

- Hypocalciuria: In Phase 1 (intensive-monitoring protocol) and Phase 2 (standard protocol), subjects will bring 24-hour urine collections to their Randomization Visit and 7-, 13-, and 19-month post-op visits. Results will be available within 1-2 days.
  - 24-hour urinary calcium < 50 mg:
    - if calcium and/or vitamin D are to be increased according to the algorithm above, no additional action;
    - if 25OHD level already at goal of 30 ng/mL and serum calcium within normal range, increase total daily calcium intake by 500 mg

Finally, renal function will be monitored closely. To receive subsequent study drug doses #2 and #3, subjects must have estimated GFR ≥ 30 mL/min/1.73 m^2, without hypocalcemia (defined as Ca <8.5 mg/dL).

Osteoporosis and excessive bone loss

At screening, potential subjects with DXA BMD T-scores of < -3.0 at the PA spine, total hip, or femoral neck will be excluded from the trial, as it may not be considered ethical to allow randomization to placebo for 18 months. Those with higher T-scores will not be excluded because the trial duration is sufficiently short and all participants will receive open-label zoledronic acid at the conclusion of the trial. Furthermore, current standard of care for bariatric surgery patients does not routinely involve perioperative antiresorptive treatment. During the trial, when DXA is performed at 7 and 13 months, if a subject's lowest T-score declines to < -3.0 at the PA spine, total hip, or femoral neck and that BMD decline exceeds the least significant change for the DXA measurement, he or she will be withdrawn from additional study drug, the DSMB will be informed, and the blind will be broken as to DMAB vs. placebo assignment. Unblinding will be done by the DSMB and a physician who is not on the study team. That physician will inform the subject of his or her treatment group assignment, counsel about risk for fracture and options for additional work-up and therapy, and, with the subject's permission, relay this information and recommendations directly to the subject's clinician.

Other denosumab or study procedure-related risks

No subject will be enrolled if they have any major dental work planned during the course of the study, and all subject complaints of thigh or groin pain will be immediately evaluated to rule out an incomplete femur fracture. The risks of phlebotomy will be minimized by careful attention to proper technique.

General considerations

If a subject is >2 weeks late for his or her study injection target date, then the PI will call the subject and counsel the subject about the importance of returning promptly. If a randomized subject decides to stop receiving study injections, the DSMB will be informed, and the blind will be broken as to DMAB vs. placebo assignment. Unblinding will be done by the DSMB and a physician who is not on the study team. That physician will inform the subject of his or her treatment group assignment, counsel about risk for fracture and options for additional work-up and therapy, and, with the subject's permission, relay this information and recommendations directly to the subject's clinician. To the extent possible, the study PIs and study staff will remain blinded to the dropout study subject's group status.

The PI at each site may end the subject's participation without the subject's consent in order to protect the health of the subject, if the subject is unable to attend to study visits, if the sponsor decides to stop the study, or due to other administrative reasons. If new information or medical problems arise during the study that may contraindicate the use of DMAB, study investigators will discuss this issue with study subjects and recommend dropout or withdrawal if needed for safety reasons. If a subject is withdrawn, the blind will be broken, and a physician who is not on the study team will counsel the subject following the procedure described above for withdrawals for severe osteoporosis and subject-initiated withdrawals. To the extent possible, the study PIs and study staff will remain blinded to the withdrawn study subject's group status.

Adverse Event Assessment and Reporting

There will be continuous safety surveillance of participants. According to the 1996 International Conference on harmonization E-6 Guidelines for Good Clinical Practice, an adverse event (AE) is defined as an untoward or unfavorable medical occurrence in a human participant, including any abnormal sign, symptom, or disease, temporally associated with participation in the research, whether or not considered related to participation in the research.

- AEs will be recorded in a systematic manner at every study visit and when otherwise volunteered by the participant. A standardized AE form will be used for recording the pertinent details.

- At the time a participant describes an AE to a study team member, the study team member may advise the participant to seek medical care, if appropriate. If the study team member is not a physician, he or she will contact the PI or another study physician to determine the best advice to provide the participant.

- AEs will be classified as expected or unexpected. An expected AE is an AE that may be reasonably anticipated to occur as a result of the study procedures or study participation and is thus described in the informed consent document. An AE will be defined as unexpected if it is not described or exceeds the nature, severity, or frequency described in the informed consent document and current IRB application.

- An AE is a serious adverse event (SAE) if it results in any of the following outcomes:
    - Death
    - Life-threatening adverse experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - Persistent or significant disability/incapacity
    - Congenital anomaly/birth defect or cancer
    - Any other experience suggesting a significant hazard, contraindication, side effect or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above
    - Event that changes the risk/benefit ratio of the study

- Cause will be determined for all AEs. The site PI (Dr. Yu or Schafer) will assess AE causality in terms of overall study participation and determine whether an AE is thought to be related to the intervention or any study-related activity. The following definitions will be used to assess the AE relationship to study participation:
    - ***Definitely Related:*** An AE is definitely related to study participation if it is clear that the event was caused by study participation. A definitely related event has a strong temporal relationship and an alternative cause is unlikely.
    - ***Probably Related:*** An AE is probably related when there is a reasonable possibility that the event is likely to have been caused by study participation. The AE has a timely relationship to the study procedure(s) and follows a known pattern of response, but a potential alternative cause may be present.
    - ***Possibly Related:*** An AE is possibly related when there is a reasonable possibility that the event might have been caused by study participation. A possibly related event may follow no known pattern of response and an alternative cause seems

more likely. In other circumstances there may be significant uncertainty about the cause of the event, or a possible relationship to study participation cannot reasonably be ruled out.

- ***Unrelated:*** The cause of the AE is known and the event is in no way related to any aspect of study participation. If there is any uncertainty regarding AE causality then the event must be assessed as possibly related to research participation and reported to the IRB as indicated. Often, the cause of an unrelated AE is disease progression.

- **Reporting:** The site PI (Dr. Yu or Dr. Schafer) will be responsible for the reporting of AEs to the site's IRB. The IRB will review any submitted reports to determine whether an event meets the definition of an Unanticipated Problem involving risks to participants or other (UP). (A UP may be an AE, although the majority of AEs are not UPs. A UP may also be another incident or outcome that suggests that the research in question places participants or others at a greater risk of harm than was previously known or recognized.) The IRB is required to report all UPs to the Department of Health and Human Services (DHHS) Office for Human Research Protection, appropriate University and affiliate officials, and study sponsors. In addition, because of the involvement of the VA in this study, the San Francisco VA Medical Center is required to report to the Office of Research Oversight (ORO) any situations that are reportable under VHA Handbook 1058.01.
  - The site PI will report AEs to the local IRB following local IRB regulations.
    - At MGH, within 5 working days or 7 calendar days of her awareness, the PI will report all unanticipated problems involving risks to subjects or others, including AEs. AEs are related or possibly related events AND serious or unexpected. Other unanticipated problems refer to any incident, experience, information, outcome, or other problem that is unexpected and related that indicates that the research places subjects or others at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized. Major protocol deviations will be reported within 5 working days of the PI's awareness, and minor deviations will be indicated in the continuing review.
    - At SFVAHCS/UCSF, within 5 working days of her awareness, the PI will report to the IRB any AEs determined to be definitely, probably, or possibly related AND Serious or Unexpected. Within 5 working days of her awareness, the PI will report to the IRB any Major Violation of protocol (including but not limited to enrollment of ineligible participant), any need for immediate protocol change to protect participant safety, and any Major Incident (including but not limited to a significant complaint or concern, lapse in study approval, or breach of confidentiality).
  - Pregnancy/lactation reports will be sent to Amgen, the IRB and the appropriate Regulatory Authorities (e.g. FDA) within 10 days of investigator awareness.
  - All other adverse events and minor protocol violations will be reported to the IRB at the time of the scheduled renewal process.
  - An annual and a final safety report will be submitted to Amgen and to the appropriate Regulatory Authorities. Additionally, suspected unexpected serious

adverse reactions will be submitted to Amgen and the appropriate Regulatory Authorities at the same time as the local IRB report.

Designation of a Data Safety Monitoring Board

In addition to the safety procedures described above, a Data Safety Monitoring Board (DSMB) will also be appointed. This joint DSMB will oversee activities at both pilot trial sites. The DSMB will be appointed before the pilot trial begins, with members selected by the PIs in consultation with the study sponsor. Members will be 2 physicians and 1 statistician, none of whom is involved in the trial. This DSMB will meet by telephone/web conference, because of the geographic distance between sites and the modest budget of this pilot trial, and will be responsible for ensuring scientific integrity and protecting the safety of study subjects. Members will meet before the trial begins and then every 6 months to review all the safety data including changes in primary and safety endpoints. In addition, the DSMB will meet when subject accrual has reached the milestones set for completion of Phase 1 (the intensive-monitoring protocol), so that the DSMB may decide whether the trial may switch to Phase 2 (the standard protocol) or whether additional intensive monitoring is necessary. Such additional intensive monitoring could take the form of continuation of Phase 1, or modification of the currently planned Phase 2 to include additional measurements or procedures. Between these regularly scheduled meetings, the PIs will notify the DSMB if any reportable AEs or Major Violations of protocol occur, as those events are reported to the IRB.

The DSMB will have access to all data and the authority to perform more formal interim analyses should there be a suggestion of increased adverse events occurring in either treated group. The DSMB will have the authority to recommend stopping the study if any safety concerns should arise. The DSMB will also review investigator and study staff performance (recruitment, retention, flow of data forms, protocol adherence, and data quality). At every semi-annual meeting and every interim contact, the DSMB will determine 1) if the risk/benefit ratio for study participation continues to be acceptable; 2) if the research protocol and informed consent document accurately and completely present risk information to research participants; and 3) whether participants already enrolled should be advised of newly identified risks.
At the semi-annual meetings and the meeting to determine Phase 1 completion, AEs and other data presented to the DSMB will be blinded to treatment group assignment. However, if the DSMB has any concerns about patterns of AEs or protocol violations, then it may request unblinded reports, which would be prepared by a statistical analyst who is not a member of the study team.

Interim Analysis

Interim analysis of efficacy data is not planned, because this is a small pilot RCT, but will be performed at the recommendation of the Data Safety Monitoring Board (DSMB, described above). Interim analysis of safety data will be performed, as described above.

Stopping Rules

This study will be stopped prior to its completion, at the advice of or in discussion with the DSMB, if any of the following criteria are met:

1. The intervention is associated with adverse effects that call into question the safety of the intervention.

2. Difficulty in study recruitment or retention is significantly impacting the ability to evaluate the study endpoints.
3. New information becomes available during the trial that necessitates stopping the trial.
4. Any situations occurs that, in the opinion of the principal investigator or DSMB, warrants stopping the trial.

Emergency unblinding
In the event of a clinical emergency, a participant will be assumed to be taking active DMAB. On an emergency basis, the blind could be broken and a participant and/or treating clinician could be informed of DMAB vs. placebo assignment.

Termination of participation
Participants may voluntarily withdraw from study participation at any time. Also, if the PI or the DSMB determines that further participation in the study or in a particular study procedure may be detrimental to the participant's health or well-being, then participation in the study will be discontinued.

XI. PUBLICATION POLICY

Publication of complete data from the study is planned. It is anticipated that the results of this study will be published in a peer reviewed scientific or medical journal and may be presented at scientific meetings. Investigators will publish results from the study in compliance with their agreement with Amgen.

XII. REFERENCES

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