Effects of Romosozumab on Bone Mineral Density, Markers of Bone Metabolism and Bone Microarchitecture in Women with Anorexia Nervosa: A Pilot Study

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I. Background/Significance

Anorexia nervosa is a serious disease leading to severe bone loss at multiple skeletal sites. The illness affects 1-2% of young women in the U.S. [1-3] and is chronic in more than 50% of affected women [4]. Bone loss is a severe, frequent and often permanent comorbid medical complication resulting in debilitating vertebral crush fractures [5]. The majority of women with anorexia nervosa have bone loss, and 50% have bone density measurements greater than 2 standard deviations below normative means [6]. It is not uncommon for bone mass in these young women to be comparable to postmenopausal women in their 7th and 8th decades [7]. Undernutrition, loss of muscle mass (which is an unavoidable consequence of weight loss) [8], and endocrine hormone dysregulation [particularly low insulin-like growth factor 1 (IGF-1)] [9] all contribute to low bone mass in women with anorexia nervosa.

Although bone density increases with weight recovery, osteopenia is often a permanent consequence of anorexia nervosa. Significant persistent osteopenia in women with anorexia nervosa has been shown in many studies and both cortical and trabecular bone mineral density (BMD) remain low more than ten years after recovery [10]. Because bone mass remains persistently low despite recovery, such women remain at a high fracture risk throughout life, estimated to be 7.1 times that of healthy women in this age range [11]. These data suggest that there may be a therapeutic window during which effective treatment should be implemented to prevent significant, lifelong bone mass reduction in this vulnerable population.

Adulthood in anorexia nervosa is characterized by a negative balance in bone metabolism, with decreased bone formation and increased bone resorption [12] (Figure 1), including lower levels of osteocalcin, a marker of bone formation, and higher levels of deoxypyridinoline (DPYRX) and N-telopeptide (NTX), markers of bone resorption, than age- and sex-matched controls (Figure 1).

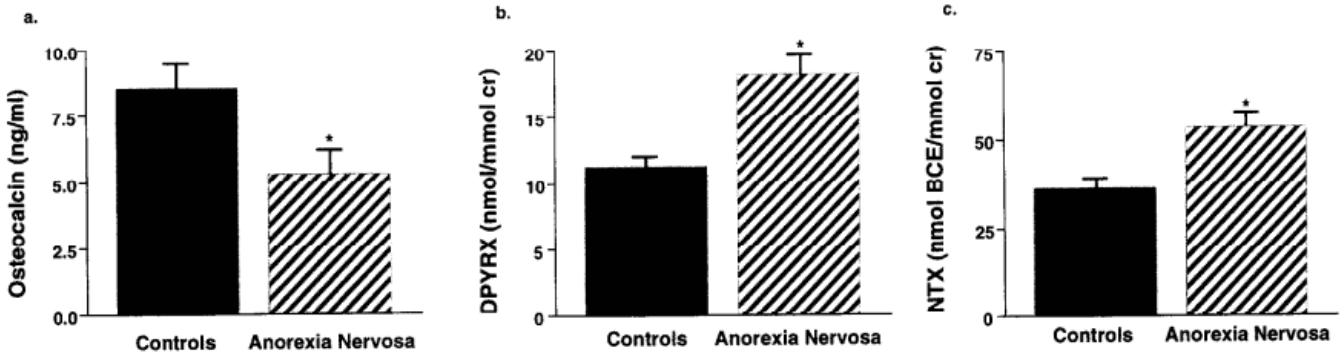


Figure 1. Levels of osteocalcin (a), a marker of bone formation, are decreased in women with anorexia nervosa compared to controls. Levels of deoxypyridinoline (DPYRX) (b) and N-telopeptide (NTX) (c), markers of bone resorption, are increased in women with anorexia nervosa compared to controls.

A number of therapies targeting the high resorptive state in women with anorexia nervosa have been studied in women with anorexia nervosa, primarily by our group. We have shown that neither low-dose estrogen at postmenopausal hormone replacement doses or oral contraceptives are effective in women with anorexia nervosa, and bisphosphonates increase bone density significantly but not to normal [13].

Agents that are anabolic to bone have been examined in pilot studies by themselves or in combination with anti-resorptives. The most promising of such agents in terms of effectiveness may be parathyroid hormone,[14] but it is not approved by the FDA for young women due to concerns about a possible risk of osteosarcoma. We have also studied low-dose recombinant insulin-like growth factor 1 (IGF-1), which is anabolic to bone and demonstrated modest increases in bone density that did not come close to normalizing bone density in women with anorexia nervosa [13], and low-dose testosterone, which was ineffective.[15] This suggests that both high bone resorption and low bone formation characteristic of women with anorexia nervosa are logical and critical therapeutic targets, but that a more potent medication such as romosozumab that addresses both the deficit in bone formation and increased bone resorption is needed. In addition, as anorexia nervosa is a psychologic illness and such patients are often overwhelmed by the requirements of therapy and the illness itself, a treatment such as romosozumab administered monthly by a healthcare clinician may have the benefit of increased compliance and therefore efficacy, as we have observed in our ongoing pilot study in which the lower frequency of Prolia administration has resulted in the lowest drop-out rate of any study in anorexia nervosa yet in our hands – and likely in anyone's. In addition, we have experienced outstanding excellent compliance to calcium and vitamin D supplementation in our ongoing pilot study of Prolia vs placebo, with no hypocalcemia. Exceptional compliance with supplementation in women with anorexia nervosa has also been reported in other studies [16].

There are no therapies that normalize BMD or that are FDA-approved for bone loss in anorexia nervosa, and in contrast to postmenopausal osteoporosis in which therapy may be life-long, the goal in patients with anorexia nervosa is treatment during the acute illness to reduce further bone loss and fracture risk. We propose that an early relatively short-term intervention with a potent combination anabolic and anti-resorptive therapy such as romosozumab during the period of rapid bone loss is an ideal, targeted therapy for the unique dysregulation of bone metabolism of young women with anorexia nervosa and may prevent significant osteopenia and reduce fracture risk in women with anorexia nervosa. We propose romosozumab therapy in a young, low-weight, population of women in which the prevalence of cardiovascular disease is extremely low [17]. Therefore, even if the possible signal implicating romosozumab in cardiovascular risk proves to have validity, this group of women would be at particularly low risk for such a complication.

II. Specific Aims

Phase 1: Romosozumab vs placebo

This [redacted]-sponsored investigator-initiated protocol is a randomized, double-blind, placebo-controlled clinical trial which aims to investigate the effect of romosozumab on BMD in women with anorexia nervosa. We will also investigate the safety of romosozumab in women with anorexia nervosa. We hypothesize that 12 months of romosozumab administration will result in an increase in bone mineral density, increase in markers of bone formation and decrease in markers of bone resorption, and improvement in bone microarchitecture in osteopenic women with anorexia nervosa compared with placebo.

Specific Aim 1: We hypothesize that 12 months of romosozumab administration will result in an increase in bone mineral density in women with anorexia nervosa with low bone mass or osteoporosis compared with placebo.

We will investigate in women with anorexia nervosa in a 12-month study whether treatment with romosozumab will increase BMD as measured by dual energy x-ray absorptiometry (DXA) compared with placebo. The primary endpoint will be change in lumbar spine BMD from baseline to 12 months. Bone density will be measured at baseline, 6 months and 12 months.

Specific Aim 2: We hypothesize that 12 months of romosozumab administration will result in a significant increase in markers of bone formation and reduction of markers of bone resorption in osteopenic women with anorexia nervosa compared with placebo.

We will investigate in women with anorexia nervosa in a 12-month study whether markers of bone formation, including osteocalcin and P1NP, will increase in the romosozumab group more than in the placebo group. We will also investigate whether markers of bone resorption, including CTx, will decrease in the romosozumab group more than in the placebo group. Markers of bone metabolism will be measured at baseline, 3 months, 6 months, 9 months and 12 months.

Specific Aim 3: In this exploratory aim, we hypothesize that 12 months of romosozumab administration will result in improved bone microarchitecture and strength in osteopenic women with anorexia nervosa compared with placebo.

We will investigate in women with anorexia nervosa in a 12-month study whether treatment with romosozumab will improve bone microarchitecture, as assessed by HR-pQCT, and improve bone strength, as estimated by microfinite element analysis compared with placebo. HR-pQCT of the tibia and radius will be performed and the following will be measured: trabecular thickness and spacing; cortical thickness and porosity; ITS (individual trabecula segmentation); finite element analysis modeling of bone strength. HR-pQCT will be measured at baseline, 6 months and 12 months.

Specific Aim 4: In this exploratory aim, we hypothesize that increases in bone mineral density will be greater in women with anorexia nervosa randomized to romosozumab who gain lean mass over 12 months compared to women randomized to romosozumab who do not gain lean mass over 12 months, and lowest in women randomized to placebo.

We will investigate in women with anorexia nervosa in a 12-month study whether increases in lean mass over 12 months are associated with a greater effect of romosozumab in increasing bone mineral density. It is known that muscle has an anabolic effect on bone through mechanical loading and muscle-bone hormonal cross-talk. Low lean body mass has been associated with impaired bone mineral density, bone microarchitecture, and estimated bone strength in women with anorexia nervosa [18]. Although studies have demonstrated that women continue to have reduced bone mineral density years after the onset of recovery from anorexia nervosa [19,20], we hypothesize that the beneficial effects of romosozumab will be greater in women who gain lean mass during the 12 months of the study. We are exploring an association between lean body mass gain and BMD gain, and not causality. Lean mass and LS BMD will be measured by DXA at 0 and 12 months.

Phase 2: Zoledronic acid

Specific Aims:

In Phase 2, participants will receive a single infusion of open-label zoledronic acid (an intravenous bisphosphonate) 5 mg after the initial 12-month administration of romosozumab or placebo. We hypothesize that 12 months of romosozumab followed by a single intravenous infusion of open-label zoledronic acid will result in a greater increase in BMD compared to 12 months of placebo followed by a single intravenous infusion of open-label zoledronic acid. Within the group of women who receive sequential therapy with 12 months of romosozumab followed by a single intravenous infusion of zoledronic acid, we hypothesize that BMD will be maintained between 12 and 24 months following administration of zoledronic acid.

Specific Aim 1: We hypothesize that 12 months of romosozumab followed by a single intravenous infusion of open-label zoledronic acid will result in a greater increase in BMD compared to 12 months of placebo followed by a single intravenous infusion of open-label zoledronic acid.

We will investigate whether sequential therapy of 12 months of romosozumab followed by a single intravenous infusion of open-label zoledronic acid results in a significantly greater increase in BMD from baseline to 24 months compared to 12 months of placebo followed by a single intravenous infusion of open-label zoledronic acid. The primary endpoint will be lumbar spine BMD at 24 months in the romosozumab/zoledronic acid group compared to the placebo/zoledronic acid group. BMD will be measured at baseline and 24 months.

Specific Aim 2: We hypothesize that within the group of women who receive sequential therapy with 12 months of romosozumab followed by a single intravenous infusion of open-label zoledronic acid, BMD will be maintained between 12 and 24 months following administration of zoledronic acid.

We will investigate whether a single intravenous infusion of open-label treatment with zoledronic acid will maintain the hypothesized gains in BMD achieved after 12 months of romosozumab administration. The primary endpoint will be lumbar spine BMD at 24 months. BMD will be measured at 12 and 24 months.

III. Subject Selection

Approximately 75 women will be screened for eligibility in order to identify 30 eligible women with anorexia nervosa, ages 20 to 60, to enroll in the study. Subjects will be randomized in a double-blind fashion to one of two groups for 12 months: monthly 1) romosozumab (210 mg, which must be administered as two injections of 105 mg) or 2) two identical placebo injections to determine effects on BMD, markers of bone metabolism, bone microarchitecture, and bone strength at trabecular and cortical sites. All subjects will meet the DSM-V criteria for AN or atypical anorexia and will be assessed for study suitability and monitored throughout the study period for their overall psychiatric clinical state in coordination with the patients' clinical care team by a study psychologist or psychiatric nurse practitioner.

Inclusion/Exclusion Criteria:

Inclusion Criteria:

- Female

- Age 20-60 years, skeletally mature with closed epiphyses
- Body mass index (BMI) ≥ 16.5 kg/m^2
- Anorexia nervosa or atypical anorexia nervosa defined by DSM-V diagnostic criteria
- BMD Z-score < -1.0
- Normal serum 25-OH vitamin D (>30 ng/mL) and calcium levels
- For women of reproductive age, agree to use an effective contraceptive method. Highly effective methods of birth control include:
    - Combined (estrogen and progestogen) hormonal methods (pills, vaginal ring, or skin patch)
    - Intrauterine device (IUD)
    - Intrauterine hormonal-releasing system (IUS)
    - Surgery to tie both fallopian tubes (bilateral tubal ligation/occlusion)
    - Woman's male partner has had a vasectomy and testing shows there is no sperm in the semen
- Dental check-up within the past year

Exclusion Criteria:

- Hospitalization within 3 months of baseline study visit, including inpatient eating disorder treatment facility; participating in a residential eating disorder treatment program within 3 months of study participation is permitted
- Myocardial infarction or stroke within 1 year preceding enrollment
- History of hypertension or use of anti-hypertensive medications within the past 6 months
- Any disease known to affect bone, including untreated thyroid dysfunction, Cushing's or renal failure
- Any medication known to affect bone metabolism within 3 months of the study, excluding oral contraceptives or other forms of estrogen administration. Bisphosphonates must have been discontinued for at least one year before participation
- Immunodeficiency or taking immunosuppressive therapy
- Serum 25-OH vitamin D level <30 ng/mL
- Serum potassium <3.0 meq/L
- Serum magnesium <1.5 meq/L
- Serum ALT >3 times upper limit of normal
- eGFR of less than 30 ml/min
- LDL > 190 mg/dL
- Hypocalcemia
- Diabetes mellitus
- Active substance abuse, including alcohol
- Current smoker
- History of malignancy
- Paget disease of bone
- Osteomalacia

- Osteonecrosis of the jaw (ONJ) or risk factor for ONJ, such as invasive dental procedures (eg, tooth extraction, dental implants, oral surgery in the past 6 months), poor oral hygiene, periodontal and/or pre-existing dental disease, and current use of corticosteroids.
- Planned invasive dental procedure over the next 24 months
- Known sensitivity to any of the products or components of the medication to be administered
- Sensitivity to calcium or vitamin D supplements
- Pregnant, planning to become pregnant within 12 months after the end of treatment and/or breastfeeding
- Subjects with a known esophageal disease
- Abnormalities of the esophagus such as stricture or achalasia
- Inability to sit/stand upright for at least 30 minutes
- Hypersensitivity to any component of zoledronic acid

Drop Criteria (conditions for withdrawing subjects). These criteria (other than pregnancy) apply until the zoledronic acid infusion has occurred:

- Pregnancy (for any subject)
- BMI < 16.0 kg/m^2 at two consecutive study visits at which weight and height are measured
- Hospitalization, including inpatient eating disorder treatment facility; participation in a residential eating disorder treatment program is permitted
- Medical instability, which will be determined by the study physician. Patients in this category will be assessed by the Data Safety Monitoring Board and continue to be assessed according to study protocol.
- Serious study–related adverse event

Source of Subjects and Recruitment Methods

To maximize enrollment of subjects with anorexia nervosa, a number of referral mechanisms have been put into place over the past years utilizing the strong relationships with directors of area eating disorder programs, as well as a regional network of health care providers -- The New England Eating Disorders Research Collaborative. Subjects will be recruited from a large population of patients with anorexia nervosa from the following locations and through the following resources:

1) New England Eating Disorders Research Collaborative was established at MGH several years ago and meets regularly for two hours each session to discuss recruitment strategies for MGH anorexia nervosa studies, review MGH research findings and discuss published data from other groups. This group represents a concerted and collaborative effort to recruit subjects with anorexia nervosa to the MGH studies and funnels patients into these studies from all over the New England area. It has been developed into an effective recruitment tool. The group includes representatives from the leadership of major eating disorder treatment centers and groups in the larger area, including: [redacted]

[redacted]

2) The Eating Disorders Clinical and Research Program (EDCRP) at Massachusetts General Hospital, which is the largest operating eating disorders clinic in New England. The Eating Disorders Unit in the Massachusetts General Hospital Child Psychiatry Service and the Adult Eating and Weight Center together form the largest outpatient Eating Disorders Clinic in the New England area. The EDCRP sends out biannual newsletters that inform clinicians and families of eating disordered patients about new research opportunities. Their web-site is a frequently hit ED site, and details about the study will be available on that site. The EDCRP networks regularly with major treatment centers for eating disorders in Connecticut, Vermont, Rhode Island, New Hampshire, and Maine and with the 250 college health centers in the Greater Boston area.

3) Walden Behavioral Care. The Neuroendocrine Unit is invited to give monthly educational talks to patients and provide information about ongoing studies at the inpatient, partial hospitalization and residential programs.

4) The Massachusetts Eating Disorders Association, which runs support groups for women with eating disorders throughout eastern Massachusetts.

5) Health services at local colleges and universities, including Boston University and Harvard University Health Services.

6) Recruitment through local university job boards, including Boston University, Northeastern University, and Harvard University

7) Social media posts (Twitter, Instagram, Facebook, etc.)

8) Mass General Brigham Rally online platform.

9) Mass General Brigham Research Study Volunteer Program (RSVP for Health).

10) Mass General Brigham Research Patient Data Registry (RPDR) and Mass General Brigham Patient Gateway. Additional recruitment will involve the Mass General Brigham Research Patient Data Registry (RPDR), which allows targeted searches of patients within the Mass General Brigham system by age, sex, and diagnosis with the ability to filter out patients by study-specific exclusion criteria. This results in a highly targeted recruitment list that allows investigators to contact potential subjects following the new OPT-OUT model. Using a targeted recruitment list from RPDR, which will exclude patients who have opted out of research invitations, we will send recruitment letters to potential subjects through Patient Gateway following the new OPT-OUT model. Additionally, we may mail recruitment letters to potential research subjects identified through RPDR who are not active on Patient Gateway.

11) Recruitment through Handshake, an online platform that connects students at universities and colleges to employers to find job opportunities.

12) Recruitment through flyers posted at local college and university campuses, community centers, and other public spaces.

13) Recruitment through a list of research subjects who have previously been recruited by our study team and who have agreed to be recontacted for future studies.

IV. Subject Enrollment

Informed consent will be obtained from all potential study subjects by a licensed physician investigator or nurse practitioner investigator before any procedures are performed. At this time, the subject will be explicitly counseled that she is free to choose whether or not to participate in this study.

Eligible subjects will be randomized at the baseline visit to the treatment or placebo group. Neither the investigator nor the subject will be aware to which group she is assigned until after the study is completed and has been un-blinded or in the case of significant safety considerations for a specific subject.

All subjects will receive a single intravenous infusion of open-label zoledronic acid at the month 12 study visit.

V. STUDY PROCEDURES

Screening Visit: A "Screening Visit" will be conducted by a member of the study clinical team to determine eligibility for the protocol. All subjects will be seen at Massachusetts General Hospital (MGH) for an outpatient visit including the following: A complete medical history including eating disorder, weight, medication, consumptive habits (smoking, alcohol, caffeinated beverages), and menstrual history will be taken. In addition, the following will be performed:

1. A clinical evaluation, including temperature, pulse, blood pressure, and a urine pregnancy test. Women will be exempt from urine pregnancy tests if they are in menopause or if they have had any well-documented method of surgical sterilization, including hysterectomy, bilateral oophorectomy, and tubal ligation. A physical and oral examination will also be performed.
2. Blood draw for comprehensive metabolic panel, CBC, magnesium, direct LDL, and 25-OH vitamin D, if not performed clinically within 6 weeks prior to the screening visit. Additional blood will be drawn to enable testing repeat in cases of lab error or tube breakage.
3. Nutritional evaluation, including weight in a gown, height, frame size, calculation of percent IBW and body mass index (BMI)
4. DXA scan of the spine, hip, radius, and total body for BMD and body composition, including lean mass, is required if the subject has not had a DXA scan within 6 months prior to the screening visit and optional if she has had one
5. Verification of the anorexia nervosa diagnosis and determination of anorexia nervosa subtype (restricting or binge/purge) using DSM-V criteria
6. Verification of lack of severe comorbid concerns (i.e. suicidality, substance use) that would preclude safe study participation will occur by the study psychologist or psychiatric nurse

practitioner. Suicidality is assessed through a verbal conversation with a psychiatric health professional. If the subject has been suicidal in the past, the psychiatric professional will ask questions to assess their level of suicidality, and whether it would be a risk to their health to partake in a study. If a subject appears to be actively suicidal, study staff will make appropriate referrals, which will often include the Acute Psychiatric Service at MGH; all efforts will be made to contact the subject's clinical treatment team.

7. Plain film of wrist/hand to determine bone age in women with primary amenorrhea who have not received estrogen for ≥1 year.
8. Electrocardiogram (ECG or EKG) to assess cardiovascular risk.

If the 25-OH vitamin D level at the screening visit is not >30 ng/ml, participants will be given one loading dose of oral vitamin D3 (cholecalciferol) 5,000 IU daily and the 25-OH vitamin D level will be checked every 3 weeks until their 25-OH vitamin D level meets the eligibility requirement (>30 ng/ml).

Baseline Visit: Eligible study subjects (n=30 women with anorexia nervosa or atypical anorexia) will be assessed at an outpatient visit at MGH for baseline testing.

1. A clinical and bionutrition evaluation and urine pregnancy test (unless exempt from urine hCG).
2. DXA scan of the spine, hip, radius, and total body for BMD and body composition, including lean mass, if screen visit did not take place within 6 weeks of baseline visit or if a DXA scan was not performed at the screening visit.
3. Baseline hormone and safety blood testing, if not performed clinically within 2 weeks prior to the baseline visit.
4. HR-pQCT and FEA of the radius and tibia
5. Eating Disorder Examination—Questionnaire
6. Eating Disorder Inventory-3
7. Paffenbarger Exercise Questionnaire
8. Calcium and vitamin D food frequency questionnaire

Randomization to treatment or placebo group will occur at the baseline visit. Twenty subjects will be randomized to active romosozumab (210 mg, which must be administered as two injections of 105 mg) and 10 subjects will be randomized to identical placebo injections. In the rare case that a subject withdraws from the study after being randomized but prior to completing the baseline visit, we will replace such dropouts with the goal that thirty subjects will complete the baseline visit. Randomization will be stratified for presence or absence of atypical anorexia nervosa, defined as BMI ≥ 18.5 kg/m2 vs. < 18.5 kg/m2. Subjects will return every month over the following 12 months to receive ongoing injections.

If screen 25-OH vitamin D level is <40 ng/ml, subjects will be given one loading dose of oral vitamin D2 (ergocalciferol) 50,000 IU at the baseline visit. All subjects will be asked to take a standard dose of 1000 mg calcium and 800 IU vitamin D supplements daily throughout the study. It is our experience from multiple clinical trials that women with anorexia nervosa are extremely compliant with calcium and vitamin D supplementation.

After the baseline visit, follow-up visits will occur at monthly intervals during the first 12 months of the study so that the romosozumab or placebo injections can be administered by a study physician, nurse practitioner, or study nurse. In rare cases where subjects are unable to travel to MGH, the study

injections may be mailed to and administered by an out-of-state, licensed physician, physician assistant, or nurse practitioner responsible for the clinical care of the study subject. A urine pregnancy test (unless exempt from urine hCG) and clinical evaluation will be performed at each monthly visit. A bionutrition evaluation, including weight in a gown, height, and calculation of percent IBW and body mass index (BMI), will be performed at months 3, 6, 9, and 12. At months 1, 3, 6, 9, and 12, blood testing, including for electrolytes (e.g. calcium), will occur unless performed clinically within 2 weeks prior to the study visit. Calcium and eGFR must be normal and 25-OH vitamin D must be > 20 ng/mL at the most recent visit in order to receive zoledronic acid at the 12-month visit. If labs are not normal, study staff will reach out to the participant's care team to assist with timely intervention with the goal that repeat labs are normal prior to the 12-month visit. Participants with serum 25-OH vitamin D ≤20 ng/ml at the 9-month visit will be given oral vitamin D2 (ergocalciferol) 50,000 IU weekly, and their 25-OH vitamin D level will be rechecked at the 11-month visit. If their repeat serum 25-OH vitamin D level at the 11-month visit is still ≤20 ng/ml, we will continue to provide oral vitamin D2 (ergocalciferol) 50,000 IU weekly, and their 25-OH vitamin D level will be rechecked every 2 weeks until their 25-OH vitamin D level meets the requirement (>20 ng/ml) to receive zoledronic acid. Additionally, participants will be given one loading dose of oral vitamin D2 (ergocalciferol) 50,000 IU at the 12-month visit if their most recent 25-OH vitamin D level is < 30 ng/ml. At months 3, 6, 9 and 12, questionnaires will be completed (EDI-3 and EDE-Q). Additionally, at months 6 and 12, a Paffenbarger exercise questionnaire will be completed. A calcium and vitamin D food frequency questionnaire will be completed at the month 12 visit as well. DXA at the spine, hip, radius and total body for BMD and body composition, including lean mass, will be performed 6 and 12 months after baseline. HR-pQCT and FEA of the radius and tibia will be performed at 6 and 12 months. Markers of bone metabolism will be measured at months 3, 6, 9 and 12.

A clinical evaluation and pregnancy test (unless exempt from urine hCG) will be performed at the 18 and 24-month visits, as will a nutrition evaluation, including weight in a gown, height, and calculation of percent IBW and body mass index (BMI) and completion of questionnaires (EDI-3, EDE-Q, and Paffenbarger exercise questionnaire). A calcium and vitamin D food frequency questionnaire will also be administered at the month 24 visit. At 18 and 24 months, markers of bone metabolism will be measured, and safety labs will be performed, unless performed clinically within 2 weeks prior to the study visit . DXA at the spine, hip, radius and total body will be performed at the 24-month study visit. HR-pQCT and FEA of the radius and tibia will also be performed at 24 months.

For the 18-month visit, subjects who do not live locally will be permitted to have their blood drawn and pregnancy test performed offsite for lab tests required for the visit. Tests that need to be run in real time will be done at local laboratories, with the results provided online to investigators. Otherwise, the samples will be mailed to investigators. Subjects who choose this option will also have an interim history performed by our staff by telephone.

Additionally, for all visits which involve questionnaires, those will be completed on paper during the visit or sent electronically via Redcap to be completed and returned to study staff.

Study Visit	Screen	Baseline	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M18	M24
Consent	X															
Clinical assessment	X	X			X			X			X			X	X	X
HCG	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Labs (CBC, magnesium, and CMP at screen; BMP, calcium, magnesium, and albumin thereafter)	X	X	X		X			X			X			X	X	X
Study drug (romosozumab) or placebo injection		X	X	X	X	X	X	X	X	X	X	X	X			
Zoledronic acid infusion														X		
Nutrition Evaluation (height, weight)	X	X			X			X			X			X	X	X
Bone Markers (CTX, P1NP, osteocalcin)		X			X			X						X	X	X
IGF-1		X						X						X		
25-OH VitD	X										X					
Direct LDL	X															
Electrocardiogram (ECG or EKG)	X															
BMD (DXA)	X	X						X						X		X
Bone Strength with FEA (Xtreme CT)		X						X						X		X
EDI-3		X			X			X			X			X	X	X
EDE-Q		X			X			X			X			X	X	X
Paffenbarger Questionnaire (exercise quantification)		X						X						X	X	X
Calcium and Vitamin D Food Frequency Questionnaire		X												X		X

Methods:

Dual-energy x-ray absorptiometry (DXA): BMD at the lumbar spine, hip, and radius, as well as lean mass and fat mass, will be assessed by DXA (Hologic 4500, Hologic, Inc., Waltham, MA) with a precision of 0.01 g/cm2 at the lumbar spine and 1.5% for lean mass [21].

Bone Microarchitecture and Micro Finite Element Analysis (μFEA): *In vitro* strength testing reflects the importance of bone morphology and microstructure in addition to BMD in determining bone strength. DXA cannot provide information about the distinct densities of the cortical and trabecular compartments of bone, the 3D-geometry of bone, or bone microstructure. HR-pQCT assesses these parameters[22] and performs *in vivo* assessment of trabecular architecture and volumetric BMD with very high resolution.[23] We will use the 2nd-generation high-resolution peripheral QCT (HR-pQCT) (XCT-II, Scanco Medical AG®, Bassersdorf, Switzerland) to measure volumetric BMD (vBMD), morphology and microarchitecture at the ultradistal radius and tibia. The variables computed by automated analysis include vBMD (g hydroxyapatite/cm^3) for the total, trabecular, and cortical regions; cortical thickness (μm) and porosity measures; and trabecular number (mm-1), thickness (μm), and separation (μm).

Measurements will be performed at the non-dominant wrist and leg (if fracture history, the non-fractured side will be used). Using a scout view, the reference line is set at the bone endplate. Scans will be at a distance from the endplate relative to the individual subject's limb length (ultradistal: 7%) to adjust for body size. Each scan includes a stack of 168 slices with an isotropic voxel size of 61 μm3. Cortical and trabecular compartments are segmented using a fully automatic contouring procedure. The fine structure will be segmented using a simple threshold process. Trabecular (trab) vBMD is calculated as the average mineral density within the trabecular region. Trab bone volume fraction is calculated directly (mineralized bone voxels/ total voxels). Trab thickness,
separation, and number are calculated using the direct 3D distance transformation method. Mean and distribution of intracortical pore diameter is measured by the direct 3D method.[24] Intracortical porosity is calculated as intracortical pore volume/total cortical compartment volume.[25] Cortical tissue mineral density is assessed after exclusion of all resolved pore space and surface voxels to minimize partial volume voxels and is a surrogate of matrix mineralization and microporosity beyond the resolution limit of the scanner.[26] **Quality control (QC) of scanner**: Included with the system (QRM, Moehrendorf, Germany) is a phantom containing rods of hydroxyapatite (densities of 0, 100, 200, 400 and 800 mg HA/cm^3) embedded in a soft-tissue equivalent resin that is scanned daily and calibrates the scanner, so that X-ray attenuation data can be converted to equivalent HA densities. Images are scored for movement artifact at acquisition (score 1-5; 1 indicating best quality)[27] and repeated for scores>2. Images scored >3 will be excluded. Weekly, a more extensive QC procedure is performed. **Microfinite element analysis (μFEA),** an established analytical engineering technique to estimate stresses and strains induced by mechanical loading of complex structures, will be used to study bone strength.[28,29] Using HR-pQCT data, it strongly predicts *in vitro* femoral and vertebral breaking strength,[30] and correlates more strongly with failure load than bone mass or structure.[28,29] *In vivo* test-retest precision is <1.0% for integral, trabecular, and cortical BMD, <2% for trabecular architecture, and <4% for μFEA stiffness and failure load. **Individual Trabeculae Segmentation (ITS):** The trabecular bone compartment is from the HR-pQCT images and will undergo ITS-based morphological analysis.[31] Volumetric decomposition will be performed to derive plate and rod bone volume fraction, number density, thickness, plate surface area, and rod length, topology: plate and rod tissue fraction, and plate–plate, plate–rod, and rod–rod junction density, and orientation, as previously reported.[31]

Laboratory testing: IGF-1 will be measured by liquid chromatography/mass spectrometry (LC/MS) (Quest Diagnostics, San Juan Capistrano, CA). Bone markers serum osteocalcin, serum C-telopeptide (CTX) and intact N-terminal propeptide of type 1 procollagen (PINP) will be measured by chemiluminescence assay at Maine Medical Center. 25-OH vitamin D will be measured by LC/MS (Quest Diagnostics, Marlborough, MA). The samples will be deidentified and coded with a unique ID that does not contain any protected health information. The study team will be able to re-identify participants, however, the investigators at Maine Medical Center and Quest will not be able to do so. Samples will be sent via air mail, following IATA shipping guidelines. Materials sent to Maine Medical Center and Quest will not be stored there for further use; extra samples will either be destroyed or sent back to the study team via air mail, following IATA shipping guidelines. If a subject wanted to withdraw their samples from either site before the samples were returned or destroyed, the study team would provide the Maine Medical Center and/or Quest with the subject's deidentified study ID, and the sample would be destroyed without further testing.

Questionnaires: Eating-disorder psychopathology will be measured using the Eating Disorder Examination-Questionnaire, a self-report measure of eating disorder severity over the past 28 days along

four dimensions-- restraint, eating concern, shape concern, and weight concern.[32] Subjects will also record current frequency of binge eating and compensatory behaviors on the EDE-Q. The Eating Disorder Inventory will be used to assess three eating-disorder-risk subscales – drive for thinness, bulimia, and body dissatisfaction.[33] Hours of vigorous physical activity per week will be quantified using the Paffenbarger scale.[34] Dietary intake of calcium and vitamin D will be quantified using a food frequency questionnaire.

VI. Biostatistical Analysis

Sample Size and Justification: Group sample sizes of 20 (active therapy) to 10 (placebo) for a total N=30 will achieve 80% power to reject the null hypothesis of equal means when the population mean difference is at least 9% with a standard deviation between groups of 8% and with a significance level of (alpha) of 0.050 using a 2-sided 2-sample equal-variance t-test. This is based on the difference in percent change in spine bone density between the romosozumab (n=65) and placebo (n=61) groups in Cosman F. *et al.* [35].

Data Analysis Plan:
The data analysis plan for the study will be as follows. The primary analysis will be intention-to-treat, with the primary endpoint being change in PA lumbar spine aBMD. Statistical significance will be defined as a two-tailed p-value of ≤ 0.05.

Phase 1 Aims 1-3: Baseline mean clinical characteristics between the 2 groups will be compared by t-test. Variables will be tested for normality using the Shapiro-Wilk test, and if non-normal, variables will be log-transformed before analysis. If the distribution remains non-normal after log-transformation, nonparametric testing using the Wilcoxon rank sum test will be performed instead. The analysis of variables at 12 months will be performed using a linear random effects model to estimate and test the effects of the 2 randomization groups (romosozumab vs placebo) from baseline to 12 months on the study endpoints.

Phase 1 Aim 4: The analysis of variables at 12 months will be performed using a linear random effects model to estimate and test the effects of the 2 randomization groups (romosozumab vs placebo) from baseline to 12 months on the study endpoints. We will also add a lean body mass x randomization group interaction term to examine if the effects of body mass change differ between romosozumab and placebo.

Phase 2 Aim 1: The analysis of variables at 24 months will be performed using a linear random effects model to estimate and test the effects of the 2 randomization groups (12 months of romosozumab followed by a single intravenous infusion of open-label zoledronic acid vs 12 months of placebo followed by a single intravenous infusion of open-label zoledronic acid) from baseline to 24 months on the study endpoints.

Phase 2 Aim 2: A secondary analysis will be performed to determine whether gains achieved in the romosozumab group are maintained through the subsequent 12-month study period using a linear random effects model with the fixed effect being time and the random effect being the intercept. Lack of statistical significance will indicate maintenance (lack of decrease--or increase) of BMD.

Phase 1 Endpoints:

Primary:
Change from baseline to 12 months in lumbar spine bone mineral density by dual energy x-ray absorptiometry (DXA).

Secondary:
Change from baseline to 12 months in bone mineral density of hip (total and femoral neck) and radius by DXA.

Change from baseline to 12 months in markers of bone metabolism: CTx, P1NP, Osteocalcin.

Exploratory:
Change from baseline to 12 months in HR-pQCT variables at the tibia and radius—trabecular thickness and spacing, and cortical thickness and porosity; change from baseline to 12 months in ITS variables; change from baseline to 12 months in finite element analysis modeling of bone strength.

Phase 2 Endpoints:

Primary:
Change from baseline to 24 months in lumbar spine BMD.

Exploratory:
Change from baseline to 24 months in markers of bone metabolism: CTx, P1NP, Osteocalcin.

VII. Risks and Discomforts

Risks from Procedures

Blood Sampling:
Blood sampling is performed in the study and there is always a very minor risk of infection, bruising, or syncope during a blood draw. There is also the discomfort of having one's blood drawn.

Bone Density and Microarchitecture Studies:
The study involves exposure to radiation from four or five DXA scans, four HR-pQCT scans, and a plain film of the wrist/hand if needed to determine bone age (only in women with primary amenorrhea who have not received estrogen for ≥1 year). The total radiation dose the subject will receive from all of the scans is 0.19 milliSievert (mSv). This amount of radiation is approximately 6% of the yearly natural background radiation from the earth and the sky (equivalent to 22 days of background radiation). There are no known health risks associated with such a dose.

Subjects will have the option to repeat scans once if there is a technical issue with the machine, which is a rare occurrence. If each scan were repeated at each visit, the total radiation dose the subject would receive from all of the scans would be no more than 0.38 mSv. This amount of radiation is equal to

about 12% of the annual background radiation from the earth and the sky (equivalent to 44 days of background radiation). Subjects have the option to refuse to be re-scanned.

All patients will have pregnancy tests (unless exempt from urine hCG) on admission prior to receiving any radiation. Subjects will also have serial pregnancy tests performed at every study visit. BMD measurements will occur five times, and HR-pQCT of the radius and tibia will be performed four times over the course of 24 months.

Risks from Medications

Oral calcium can cause constipation and a metallic taste in the mouth.

Vitamin D in the doses being administered does not result in any side effects.

Romosozumab Administration:
Two separate subcutaneous injections are needed to administer the total dose of romosozumab 210 mg. A study clinician will inject two syringes, one after the other, every month for 12 doses in the abdomen, thigh, or upper arm.

Romosozumab 210 mg may cause all, some, or none of the side effects listed below. These side effects can be mild but could also be serious, life-threatening, or even result in death. In addition, it is possible that a subject may experience an allergic reaction that has not been seen before.

Side effects that other people with osteoporosis or bone loss have had in research studies that are thought to have been caused by romosozumab 210 mg are:

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

Common side effects (which may affect between 1 and 10 people in every 100):
- headache
- muscle spasm
- swelling in extremity
- lack of energy
- neck pain
- trouble sleeping
- tingling or numbness in extremity

Uncommon side effects (which may affect between 1 and 10 people in every 1,000):
- allergic reaction (drug hypersensitivity) (see description below)
- hypocalcemia (see description below)
- heart attack or stroke (in postmenopausal women) (see description below)

Rare side effects (which may affect between 1 and 10 people in every 10,000):

- osteonecrosis of the jaw (ONJ) (see description below)
- unusual thigh bone fracture (atypical femoral fractures) (see description below)

Hypersensitivity reactions. Hypersensitivity reactions, including angioedema, erythema multiforme, dermatitis, rash, and urticaria have occurred in romosozumab-treated patients. Subjects will be instructed to call the study doctor immediately if they are concerned about an allergic reaction, and to go immediately to the emergency room if they are concerned about a severe allergic reaction. If an anaphylactic or other clinically significant allergic reaction occurs, romosozumab will be discontinued and the subject will be referred for appropriate treatment.

Hypocalcemia. Transient hypocalcemia has been observed in patients receiving romosozumab. Subjects with hypocalcemia, a 25-OH vitamin D level <30 ng/mL, or eGFR <30 ml/min will be excluded from participation in the study. Subjects will be monitored for signs and symptoms of hypocalcemia. All subjects will take daily calcium and vitamin D while in the study.

Major adverse cardiac event. In a randomized controlled trial in postmenopausal women, there was a higher rate of major adverse cardiac events (MACE), a composite endpoint of cardiovascular death, nonfatal myocardial infarction and nonfatal stroke, in patients treated with romosozumab compared to those treated with alendronate. Subjects who have had a myocardial infarction of stroke within the preceding year will be excluded from participation in the study.

Osteonecrosis of the jaw (ONJ). ONJ is a potentially serious condition that can present as a sore in the mouth through which the jaw bone is sometimes visible. The gum tissue over the bone may heal slowly or not heal at all. How this happens is poorly understood. The symptoms of ONJ include pain or infection in the jaw bone and gums. Subjects will be instructed that if they develop any of these symptoms, they should see their doctor who should examine her mouth to determine if she has ONJ. The risk of developing ONJ is higher in patients who have had tooth removal, gum surgery, infections in the mouth, dental implants or other dental procedures. Subjects will be instructed that it is important that they maintain good oral hygiene and avoid dental procedures immediately before and during their participation in the study, if possible. They will also be instructed that their study doctor and dentist should discuss the benefits and risks of any dental procedure. Additionally, they will be instructed to tell their dentist that they are taking romosozumab before a dental procedure is planned. Although ONJ has been reported rarely in patients receiving romosozumab, we will take all possible precautions to prevent it from occurring, specifically, we will exclude all subjects with an invasive dental procedure within 6 months prior to the baseline visit or planned dental procedure over the next 6 months, and will require all subjects to have had a dental exam within 1 year prior to enrolling in the study.

Atypical femoral fractures. An atypical femoral fracture is an unusual thigh bone fracture that may occur with little or no trauma. Fractures can occur in both thighs at the same time. Atypical fractures have been reported rarely in patients receiving romosozumab. It is unknown whether the risk of atypical femoral fracture continues after stopping therapy. It can present with new or unusual hip, thigh, or groin pain weeks to months before the fracture is diagnosed. Subjects will be instructed that if they develop any of these symptoms, they should report them to the study doctor and the physician they see for clinical care. Patients presenting with an atypical femur fracture will also be assessed for symptoms and signs of fracture in the contralateral limb.

In clinical studies, after romosozumab discontinuation, BMD returns to approximately baseline levels within 12 months in the absence of follow-up antiresorptive therapy. This is why we propose to follow the 12 months of romosozumab or placebo with a single intravenous infusion of open-label zoledronic acid at the 12-month visit.

The potential effects of romosozumab on the fetus are not known, and therefore precautions against administration to pregnant patients will be instituted. All patients will have pregnancy tests on admission prior to receiving study medication and serial pregnancy tests at every study visit and will be required to use contraception in order to participate in the protocol (unless exempt). Any subject who becomes pregnant during the course of the study and is discharged from participation, will undergo a follow-up study visit. This will include assessment by an MD or NP for study medication related side effects, including hypocalcemia, and measurement of a calcium level if it has not been done clinically.

After patients start taking romosozumab, it is possible that their body may make antibodies to romosozumab. Antibodies to romosozumab have not been associated with changes in the efficacy or safety of romosozumab.

Zoledronic acid Administration:

The risks of zoledronic acid therapy are as follows: flu-like symptoms (low-grade fever, fatigue, nausea, muscle aches, joint aches, headache), skin rash, diarrhea, and joint pain. Less common side effects include abdominal or stomach pain; renal impairment and acute renal failure; atrial fibrillation; hypocalcemia; stomach cramping; belching; severe bone, joint, and muscle pain; hypertension; blurred vision or change in vision; chest pain; constipation; cough; dizziness; dry eyes; fever; general feeling of discomfort or illness; leg cramps; nausea; ringing in the ears; swelling of the feet or lower legs; and weakness. On rare occasion, red sore eyes have been reported among those on zoledronic acid therapy.

Two very rare potential complications of bisphosphonate therapy are osteonecrosis of the jaw and atypical femur fractures. Osteonecrosis is a bone condition that results from poor blood supply to an area of bone causing bone death. Although it can affect any bone, only osteonecrosis of the jaw has been found to coincide with bisphosphonate therapy. The vast majority of cases have developed in patients treated with high dose intravenous therapy (94%), and with multiple myeloma or metastatic breast cancer (85%). In the majority of cases of jaw osteonecrosis in the treatment of osteoporosis, dental surgery and/or trauma to the jaw were preceding factors in the development of osteonecrosis of the jaw. The risk of jaw osteonecrosis increases with the length of time one is exposed to intravenous bisphosphonate therapy. The skeleton is constantly developing micro fractures from skeletal loading. These micro fractures are repaired by the normal process of bone remodeling, which requires bone resorption. Bisphosphonates suppress bone remodeling and remain in the skeleton for many years. Long term bisphosphonate use may cause accumulation of micro fractures, thereby increasing skeletal fragility. Atypical subtrochanteric femur fractures are fractures in the bone just below the hip joint. Diaphyseal femur fractures occur in the long part of the thigh bone. These fractures are very uncommon and appear to account for less than 1% of all hip and femur fractures overall. Although it is not clear if bisphosphonates are the cause, these unusual femur fractures have been predominantly reported in patients taking bisphosphonates long term.

Flu-like symptoms can occur in persons receiving intravenously administered bisphosphonates. These flu-like symptoms may include fever, chills, fatigue, headache and nausea, and typically resolve within 2 days. Participants will be advised that they may take over-the-counter acetaminophen 650-1000 mg PO once on the morning of the zoledronic acid infusion as prophylaxis for these symptoms. As bisphosphonates remain in the bones for many years after administration has concluded, it remains unclear as to whether bisphosphonates are released from bone during pregnancy or breastfeeding. Published reports to date have not demonstrated increased birth defects in infants born to women receiving bisphosphonates before or during pregnancy. Study participation by pregnant or breastfeeding women is not allowed and is a study exclusion.

Although the long-term risk of zoledronic acid in young women is unknown, bisphosphonates have been used in premenopausal women to treat osteopenia due to glucocorticoids. In addition, these medications are used widely in the community and it is essential that their efficacy be assessed.

A study psychologist or psychiatric nurse practitioner will review all screened subjects, conduct a psychiatric interview at baseline and at any other time the staff requests. S/he will continue to monitor and ensure the ongoing psychiatric care of patients with their primary clinical psychiatrists and therapy teams and also provide individual consultation to subjects as needed. All patients recruited will continue to receive medical and psychiatric care from their treatment teams and all participants must have a clinical provider in place. A study psychologist or psychiatric nurse practitioner will continue to work closely with the patients' clinical care treatment teams to ensure patient safety and continued appropriateness for the study from a psychiatric perspective. In summary, the study psychologist or psychiatric nurse practitioner involvement will be to provide a comprehensive psychiatric evaluation of potential study subjects, provide ongoing psychiatric monitoring of patients during the course of the study, and, be available by page for any questions that arise from study subjects, other investigators, and the patients' clinical treatment teams and psychiatric providers. S/he will review any specific psychiatric issues regarding patients involved in the protocol with the other investigators, review data and participate in all aspects of the proposed study as a key member of the research team. S/he will attend Data Safety Monitoring Board meetings and will, together with the P.I., keep the Data Safety Monitoring updated on any issues that impact patient safety.

Physician Availability: A physician will be available by pager at all times during the study to answer any questions or address any concerns that a subject may have. All efforts will be made to protect the confidentiality of study subjects who will be referred to by an enrollment number only.

Patient Confidentiality: Confidentiality of the patients will always be of paramount importance to study investigators. All data on patients will be kept in confidential study binders and electronic files accessed by only study investigators and study staff. No study subject data will be shared with persons other than those directly involved in the study, except at the documented request of the subject.

VIII. Potential Benefits

Each subject will have a complete nutritional analysis and BMD performed. These data, including relative fracture risk and total calcium intake, will be made available to the subject. In addition, a physical exam and blood testing will be performed in all subjects, and these data will also be made available to the subject.

The subject's bone mineral density may increase during the study, although if it does increase, it is not known how long it will remain higher. We hope and anticipate that the information learned from this study will allow us to better understand the pathophysiology of bone loss in anorexia nervosa and to improve the treatment of patients with bone loss associated with under-nutrition.

IX. Monitoring and Quality Assurance

No study procedures will be performed prior to approval by the Mass General Brigham Human Research Committee (IRB). Furthermore, all changes to the protocol or consent form will be submitted to the Human Research Committee for approval before being implemented. No data on study subjects will be shared with persons other than those directly involved in the study, except at the documented request of the study subject. Data obtained from each subject's visit will be reviewed by the investigators weekly. Study staff will review the completeness of case report form (CRF) entries, source documents, and informed consent during and immediately following the visit.
All adverse events, collected at all study visits, will be reported to the Human Research Committee (IRB) according to Mass General Brigham adverse event Reporting Policy and to the FDA according to Title 21 of the Code of Federal Regulations. A study-related adverse event will be considered serious if it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Additionally, any medical event that may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed. All adverse events will be reported to the Data Safety Monitoring Board every 6 months, and this report will be submitted to the IRB every 6 months. Safety data will also be reported to [redacted] as follows:

> Suspected Unexpected Serious Adverse Reaction (SUSARs) will be reported to [redacted] at the time of regulatory submission. Pregnancy/Lactation exposure and any associated reports/outcomes (i.e. unexpected pregnancy, pregnancy of partner, spontaneous abortion, congenital anomaly etc.) will be reported within 1 business day of sponsor awareness, for reports meeting serious criteria. Reporting to [redacted] will not exceed 15 calendar days of sponsor awareness for non-serious reports.

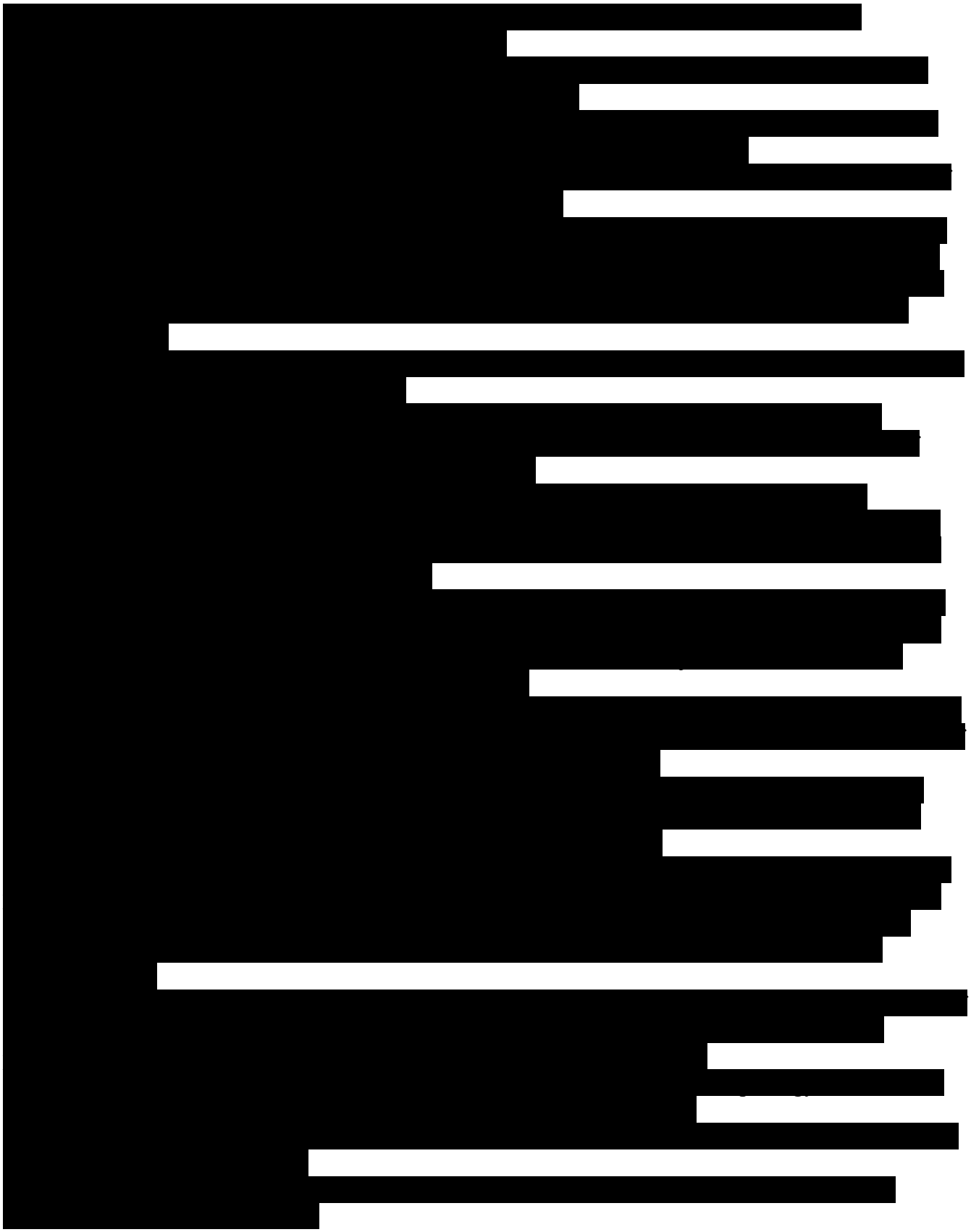
Data and Safety Monitoring
A Data Safety Monitoring Board will be established at Massachusetts General Hospital consisting of five members -- the following five are staff members at Massachusetts General Hospital -- [redacted]

The Data Safety Monitoring Board will meet twice a year after study activation, or more often as needed. The Board will review all study procedures, all adverse events, study violations, exceptions and deviations as well as study inclusion and exclusion criteria. The Board will review all safety monitoring blood tests and study data as available. The data reviewed by the Data Safety Monitoring Board will be

blinded as to randomization assignment, except in cases in which the Data Safety Monitoring Board or study investigators feel that unblended data should be reviewed due to safety concerns. All study related serious adverse events will be reported to the Board within 24h of occurrence. Based on recruitment, review of adverse events and other parameters to be established by the Board, the Board may request an interim analysis or request unblinding or halting of the study for safety concerns or other issues that impact on safety.

References

[redacted]



[redacted]