A PHASE II, SINGLE ARM CLINICAL STUDY OF DENOSUMAB EFFECT ON BREAST DENSITY AND BREAST TISSUE BIOMARKERS

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Amendment number	Protocol version	Version Date	Description
1	5	10/20/2015	Updated “Risks and Discomforts” section; removed cellulitis and edited the frequency of osteonecrosis of the jaw
2	6	12/18/2015	Modified the definition of menopause as “history of amenorrhea for at least one year or hormone levels (estradiol/FSH) consistent with postmenopausal status if post-hysterectomy status, or history of surgical/medical castration”; removed requirement of dentist visit to grant eligibility.
3	7	4/29/2016	Minor changes and clarifications to the Schedule of Events; introduced the “Breast and Bone Health” questionnaire; clarified validity of bloodwork for eligibility purposes; section 8.1 “information on study agent” updated with new published efficacy and safety results; new UH research budget form
4	8	10/28/2016	Removed osteopenia (T score < -1) as eligibility requirement; minor changes to the schedule of events (blood chemistry done at BL2, 4th injection and follow up visit)
5	9	12/19/2016	Title changed t o “A Phase II, single arm clinical study of denosumab effect on breast density and breast tissue biomarkers”; section 27.2 is updated to reflect that we expect to complete the study with at least 20 evaluable women per group
6	10	5/1/2017	Various risks and discomforts updates following Amgen release of yearly safety update; clarify that patients do not need calcium and/or vitamin D supplements if respective blood values are within range; increased overall payment to study subjects to max $250.
7	11	7/14/2017	Reduced number of blood investigations and assigned calcium and vitamin D to research budget
8	12	4/26/2018	Added patients on long term care with aromatase inhibitors to the study population; small edits to the analytical plan (no ADC or DW-MRI); specified blood investigations required to ascertain menopausal status in women who underwent surgical removal of 1 or both ovaries in the previous 12 months; added a urine dip-stick pregnancy test before each injection for women of reproductive potential. CTCAE v5 now in use for AEs

9	13	6/18/2018	Edited eligibility section to specify that women at elevated risk of developing breast cancer (e.g. hyperplasia or genetic risk) are not eligible for the study
10	14	9/11/2018	Removed the 3-month observation period. Reduced MRI schedule from 4 to 2 MRIs in total. Remove blood calcium STAT labs for injections 3, 4, 5 and 6. Dmab study injections can be administered at home by a trained nurse, unless risk factors for adverse reactions are present or low calcium. Range of follicular phase for premenopausal subjects is now defined as up to 14 days from the start of the period. Revised primary hypothesis and power estimation. Increased period of validity for baseline bloodwork. Updated risk of ONJ (section 10.1).
11	15	5/22/2019	Added exclusion criteria: thyroid/parathyroid surgery, malabsorption syndromes, hypoparathyroidism. Added additional calcium testing at injection #3 and 2 months (8 weeks) post last injection.
12	16	06/27/2019	Shortened washout period from chemotherapy/radiation from 3 to 1 months
13	17	12/20/2019	Removed enrollment limitations relative to menopausal status
14	18	05/05/2020	Removed requirement to document temperature in eCRFs during research visits. During COVID pandemic, will continue to obtain temperature readings per clinical guidelines prior to administering any study drug at home visit or at cancer center. Will only treat afebrile subjects, but as temperature is not a safety test or an endpoint needed to reach study objectives will not document this result in eCRFs.
15	19	01/05/2021	Edits to accommodate investigator Dr. Patricia Thompson' moving her lab to Cedars Sinai (CA). Adding section "18.1 COVID-19 risk mitigation and exceptions". Edited section 28.4 to address data sharing modality with Cedars-Sinai (page 21)

2. CONTACT INFORMATION

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4. PROTOCOL OVERVIEW

This is a non-randomized phase II trial of denosumab at 120 mg subcutaneous injection monthly in pre- and post-menopausal women diagnosed with stage 0-III breast cancer who are currently in long term follow up care. Eligible participants may or may not be receiving anticancer treatment with aromatase inhibitors (either exemestane, letrozole or anastrozole), but must have completed all planned chemotherapy, or radiation for at least 1 month. All patients will undergo quantification of breast density by MRI at baseline and after 6 months on denosumab. This is an investigator-initiated trial and Amgen will provide the trial drug, Xgeva®.

The primary endpoint of the study will be the change in breast density of the contralateral, uninvolved breast as measured by quantitative Fat Water Ratio mapping (FWR-MRI) comparing 6 months on denosumab (MRI_{EOS}) to breast density by FWR-MRI at baseline (MRI_{BL}). As changes in breast density in the *contralateral, uninvolved breast* will be the primary endpoint of the study, patients with bilateral breast cancer, bilateral mastectomies, or extensive breast implant in the contralateral breast will be ineligible.

The following are planned exploratory endpoints.

1) Change in tissue composition (epithelium, stroma and fat) after 6 months on denosumab as determined by paired biopsies performed pre and post denosumab therapy
2) Change in specific and global gene expression patterns in breast biopsy tissues after 6 months on denosumab
3) Change in progestogen levels and the OPG/RANKL ratio after 6 months on denosumab
4) Circulating progestogen levels and the OPG/RANKL ratio will also be correlated to MRI-acquired fat-to-water ratio at baseline and after 6 months of denosumab treatment.

For the tissue biomarkers, core needle biopsies will be obtained in a subset of women who consent to the optional procedure from the uninvolved contralateral breast at baseline and after 6 months of denosumab therapy. It is anticipated that ~75% women at baseline will provide tissue samples for cross sectional comparative analyses with MRI features at baseline and ~60% after 6 months on denosumab will agree to a second biopsy to provide tissue to conduct analyses of biomarker response to intervention. Tissue studies will include characterization of tissue histology (graded by cellularity and stromal elements) and molecular measures of proliferation and apoptosis. The attached schedule of events depicts the study schema and primary measurements to be conducted during the trial (Appendices 1.1 and 1.2).

5. STUDY RATIONALE AND BACKGROUND

5.1. Women still die from ER+ breast cancers

Each year, more than 192,000 incident and 40,000 breast cancer deaths are recorded among women in the U.S. (1). Aromatase inhibitors (AIs) and selective ER modulators (i.e., tamoxifen) are effective in delaying or preventing the progression of ER+ disease in 50-70% of women diagnosed with ER+ tumors (2). AIs demonstrate modest, but consistently superior, efficacy for reducing risk of breast cancer events in postmenopausal women and are now the primary modality for adjuvant hormonal therapy in these patients (3). This success provides the proof of principle that breast cancer is a preventable disease. In spite of significant success with hormone-targeted therapies and improvements in selective use of chemotherapy, breast cancer remains a major cause of death in women. This is partly due to the poor risk benefit ratio for the SERMS and AIs for primary prevention and the fact that there are currently no drug options for the prevention of ER negative disease, which tends to be more aggressive with early metastasis.

5.2. Targeting RANKL for the prevention of breast cancer

There is now convincing evidence that extensive areas of radiographically-dense tissue in the breast is an independent risk factor for the development of breast cancer (4-9). The presence of dense tissue that occupies

more than 50 percent of the area of a mammographic image, which occurs in up to 30% of postmenopausal women, is associated with a three- to five-fold elevated risk of breast cancer. While strongly associated, the biological or mechanistic link between breast density and breast cancer risk has not been elucidated. Of the factors that have been studied, menopause has the most profound effect on breast density with dramatic decreases observed in the 12 months following last menstruation. Progesterone as medroxyprogesterone acetate in combination with estrogen has a greater effect on breast density than estrogen only hormone therapy with decreases in breast density with agent withdrawal more evident with cessation of MPA + E than E alone. (Colacurci, Fornaro et al., Hofseth, Raafat et al. 1999) Postmenopausal hormone therapy, particularly combination therapies that include a progesterone component and, in particular, medroxyprogesterone acetate (MPA) prevent the decline in breast density and promote an increase in women with low breast density. The effect of progesterone on the breast stroma and epithelial compartments, which collectively make up dense appearing tissue on a mammogram, are hypothesized, in part, to explain the higher risk of breast cancer among users of combination hormone therapy for menopausal symptoms. (Hofseth, Raafat et al. 1999, Campagnoli, Clavel-Chapelon et al. 2005, Narod 2011, Obr and Edwards 2012).

More recently, it has been shown that progesterone upregulates the expression of RANK ligand in the breast and that by doing so is thought to act in the promotion of breast epithelial stem cells. (Obr and Edwards 2012) This effect has been postulated to explain the association between progesterone and breast cancer (see rationale for drug selection). A number of factors have been shown to modulate the levels of RANK ligand, which is now hypothesized to act in the breast to promote the growth of mammary stem cells. Here, we postulate that it is RANK ligand that mediates the association between breast density and risk of breast cancer for which progesterone is one of several factors that influence the availability of RANK ligand in mammary tissue to promote or sustain higher breast density. As such, we hypothesize that inhibition of RANK ligand with the anti-RANK ligand antibody therapeutic agent denosumab will decrease breast density, a risk factor for breast cancer development.

5.3. Overall significance of the study

This study will determine if therapeutic suppression of RANK ligand has a direct effect on breast tissue that is detectable as a decrease in breast density. We will investigate the effect of denosumab on breast tissue using novel imaging approaches discussed below. Secondarily, we will explore whether denosumab has direct effects on serum or breast tissue levels of OPG and RANK ligand gene expression and whether or not change in expression patterns correlates with change in breast density.

6. STUDY HYPOTHESIS AND OBJECTIVES

Our primary objective is to assess the effect of denosumab on breast density in pre and post-menopausal early stage breast cancer patients. Denosumab will be administered at 120 mg subcutaneously monthly for 6 months to pre and postmenopausal breast cancer patients, respectively with breast density assessed by magnetic resonance fat water imaging (FWR-MRI) of the breast. We will test the primary hypothesis that RANK ligand inhibition by denosumab (Xgeva) given 120mg q monthly will significantly decrease breast density over 6 months. The change in breast density from the baseline MRI (MRI_{BL}) to the second MRI at the end of study visit (MRI_{EOS}) (1 month after receiving the last denosumab study injection) will provide breast density measurements for determining change in breast density in women on intervention for 6 months (**the primary objective)**.

6.1. Primary Objective:

A comparison of the change in breast density as measured by MRI-acquired fat-to-water ratio (FWR-MRI) in the *contralateral, unaffected* breast pre- and post-denosumab (primary trial endpoint).
Hypothesis: At least 30% of women treated with denosumab will show a $\geq$ 5% relative decrease in breast density after 6 months denosumab treatment when compared to their baseline density.

6.2. Planned Exploratory Objectives:

i. Correlate baseline tissue characteristics (*e.g.,* cellularity) and tissue biomarkers (*e.g.*, Ki67, OPG, RANKL) to FWR-MRI to identify tissue correlates of MR image features at baseline.

ii. Explore change in mammary gland tissue architecture, cellularity and proliferation/apoptotic index in women consenting to serial biopsy pre and post-denosumab.
iii. Collect and bank urine and plasma for biomarker studies of denosumab effect on circulating OPG and RANKL concentrations and their ratio to each other.
iv. Collect and bank plasma for biomarker studies correlating hormone levels to baseline breast density
v. Assess the levels of progesterone before and after denosumab treatment

7. PARTICIPANT POPULATION

Participants will be pre- and postmenopausal/perimenopausal women with early stage (stage 0-III) breast cancer, including having received biopsies for ductal or lobular carcinoma *in situ*, who are disease free at enrollment and who are at least 1 month post treatments including radiation, surgery and chemotherapy. Women may or may not be on long-term hormonal therapy with aromatase inhibitors (e.g. letrozole, exemestane or anastrozole). We estimate a 10 % dropout rate prior to study conclusion (6 months imaging endpoints) for a total of 40 fully evaluable participants. As change in breast density is the primary outcome of the study, women are required to have a modifiable amount of breast density at baseline, based on mammographic evaluation within the last year. Thus, women with breast density in the unaffected breast described as scattered fibroglandular tissue, or greater, on mammography are eligible. Women with greater breast density (i.e. typically described as heterogeneously dense, mostly dense, etc. on mammography) are also eligible. The PI can evaluate the most recent mammograms and adjudicate the density for eligibility.

8. INFORMATION ON STUDY AGENTS

8.1 Denosumab

Denosumab is a fully human monoclonal IgG2 antibody that binds with high affinity and specificity to RANK ligand (Kd 3 x 10^{-12} M). This binding prevents the activation of RANK and inhibits the formation, activation, and survival of osteoclasts, the result of which is a reduction in the number and function of osteoclasts and, consequently, a decrease in bone resorption and an increase in cortical and trabecular bone mass, volume, and strength (10). Denosumab is highly specific and binds only to RANK ligand and no other members of the tumor necrosis factor (TNF) family, including TNFα, TNFβ, TNF-related apoptosis-inducing ligand, or CD40 ligand (Elliott et al, 2006).

The safety and efficacy of denosumab for the prevention of skeletal-related events in patients with bone metastases from solid tumors was demonstrated in three international, randomized (1:1), double-blind, active-controlled, non-inferiority trials comparing denosumab with zoledronic acid. In all three trials, patients were randomized to receive 120 mg denosumab subcutaneously every 4 weeks or 4 mg zoledronic acid intravenously (IV) every 4 weeks (dose adjusted for reduced renal function). Patients with creatinine clearance less than 30 mL/min were excluded. In each trial, the main outcome measure was demonstration of noninferiority to time to first skeletal-related event (SRE) as compared to zoledronic acid. The breast cancer trial enrolled 2046 patients with advanced breast cancer and bone metastasis. Randomization was stratified by a history of prior SRE (yes or no), receipt of chemotherapy within 6 weeks prior to randomization (yes or no), prior oral bisphosphonate use (yes or no), and region (Japan or other countries). Forty percent of patients had a previous SRE, 40% received chemotherapy within 6 weeks prior to randomization, and 5% received prior oral bisphosphonates. Median age was 57 years, 80% of patients were White, and 99% of patients were women. The median number of doses administered was 18 for denosumab and 17 for zoledronic acid. Denosumab delayed the time to first SRE by 18% and first or subsequent SRE by 23%. Overall survival and progression-free survival were similar between arms in all three trials.

Recently published results from a placebo-controlled trial, involving 3425 postmenopausal, women with early-stage HR-positive breast cancer currently receiving aromatase inhibitor therapy, confirmed that adjuvant denosumab reduced the risk of clinical fractures in this population, irrespective of their bone density status, at

baseline (11). This study included over 900 patients treated with denosumab with normal bone mineral density at baseline who equally benefited from denosumab therapy compared to patients with osteopenia at baseline. More importantly, the same study found that after a median follow-up of four years, patients assigned to denosumab had an 18% reduced risk of disease recurrence or death compared with those assigned placebo (12). The ongoing double-blind placebo-controlled trial D-CARE (NCT01077154) randomized 4,509 women with stage II or III breast cancer to either denosumab 120mg monthly x 6 months (same regimen as in this study) and then continued therapy with q 3 months of Xgeva or a placebo for up to 5 years of treatment. Bone mineral density was not obtained at baseline and low bone mineral density was not an inclusion critieria. From the population treated, it can be assumed that ~40-50% of the women who received denosumab treatment likely had normal bone density at baseline. Similarly, it can be assumed that a large fraction (~40-45%) of participants were pre-menopausal at accrual. The study started accrual in 2010 and reached its accrual goals in 2016. Thus, all participants (over 2,250 women) randomized to denosumab received the agent for at least 6 months, and most have completed the 5 years of therapy. The study is reviewed for safety every 6 months by an external data and safety monitoring committee and no unusual safety signals have been reported with the study continuing as per protocol without revision.

Denosumab is FDA approved for prevention of skeletal-related events in patients with bone metastases from solid tumors, however its use in this protocol is investigational. The FDA has recognized an IND exemption for the use of denosumab in this study, in accordance to current regulations [21 CFR 312.2(b)].

9. RATIONALE FOR DRUG AND DOSE SELECTION

RANK may be expressed in human breast tumors and functional on breast cancer cells (13). Signaling through RANK/RANKL regulates the development of the mouse mammary gland during pregnancy. Transgenic mice overexpressing murine RANK exhibit increased mammary tumorigenesis relative to wild type mice in both induced (medroxyprogesterone acetate [MPA] + 1,7-dimethyl-benz(a) anthracene [DMBA]) and spontaneous (multiple pregnancies) models of mammary tumorigenesis (14). RANK expression is associated with an increased incidence of more extensive ductal hyperplasia and mammary intraepithelial neoplasia, an increased incidence of adenocarcinoma, as well as a shorter latency to tumor formation and increased numbers of tumors per gland in mouse (15). Conversely, inhibition of the RANK/RANKL axis reduces hormone-induced mammary epithelial proliferation and reduces the incidence of mammary tumors (14, 16). These observations support the hypothesis that inhibition of RANKL with denosumab may delay or prevent the development of breast cancer and/or disease recurrence in early-stage breast cancer.

Based on safety, pharmacokinetics and pharmacodynamic data from phase I, II and III studies, a dosing regimen of denosumab 120 mg SC Q4W for 6 months is proposed. Specifically, Study 20040113 tested five different dosing schedules of SC denosumab versus IV bisphosphonates in 255 patients with breast cancer and bone metastases: 30 mg, 120 mg or 180 mg SC given Q4W, and 60 mg or 180 mg given Q12W (17, 18). The regimen of 120 mg Q4W was associated with the greatest median reduction of the bone turnover marker, uNTX, at week 13, the primary endpoint of the study. Unlike lower doses, a 120 mg Q4W schedule avoids low drug exposures, which could potentially translate into insufficient efficacy in some patients. Denosumab was found to be safe and well tolerated in all study arms. As the duration of the study is only 6 months, long term and rare complications of denosumab therapy including ONJ and/or atypical fractures would be unexpected to occur. In addition, treating at the maximal efficacious, yet safe dose of the drug will maximize our probability of discovering changes in breast density and/or tissue markers of breast risk that would foster the development of larger and longer duration trials of denosumab for preventing breast cancer recurrence.

Additionally, over 2,250 early stage breast cancer patients have been randomized to receive denosumab at 120mg SC q month x 6 and then 120mg SC every 3 months for the next 4 and a half years (NCT01077154 clinical trials.gov). In this trial, decreased bone density was not an eligibility criteria and it can safely be assumed that many women (~40-50%) enrolled on this study had normal bone mineral density treated with this dose intensive schedule of denosumab. The trial completed accrual (study was enrolling patients from 2010-2016) and is in follow-up for the primary endpoint of bone metastases free survival (expected 2017). Previously, denosumab was found to be superior to other bisphosphonates (zoledronic acid) in preventing skeletal-related

events in cancer patients with bone metastases, including breast, again irrespective of bone density (19). In this trial as well, no unexpected toxicity to denosumab was observed compared to zoledronic acid and the trial was never stopped or placed on hold by the external DSMB.

Increasing evidence suggests that bone density at baseline does not alter the risk profile for short-term denosumab treatment. For this reason, we plan to enroll women regardless of their bone density in this trial involving 6 months of Denosumab therapy. However, we will continue to collect information on bone density at baseline, via DEXA scan as patients with low bone mineral density will receive known benefit from therapy.

Prolia (denosumab 60mg Q6mo) is commonly prescribed to patients in long term follow up care for breast cancer, especially those with hormone receptor positive disease and receiving anti-estrogen therapy. Treatment with aromatase inhibitors (anastrozole, exemestane, letrozole) does not alter breast density and does not increase the risk of side effects.

10. TOXICITY FOR DENOSUMAB REPORTED ADVERSE EVENTS AND IDENTIFIED RISKS

The safety of Xgeva was evaluated in three randomized, double-blind, active controlled trials comparing denosumab to zoledronic acid for the prevention of skeletal related events in patients with bone metastasis from prostate cancer, breast cancer, or other solid tumors, or lytic bony lesions from multiple myeloma. A total of 2841 patients received at least one dose of Xgeva (Appendix 6). The most common side effects attributable to denosumab were hypophosphatemia and hypocalcemia. Severe hyposphosphatemia (serum phosphorus less than 2 mg/dL) occurred in 15.4% of patients on denosumab compared to 7.4% of patients treated with the intravenous bisphosphonate zoledronic acid. Severe hypocalcemia (corrected serum calcium less than 7 mg/dL) occurred in 3.1% of patients treated with denosumab compared to 1.3% of patients treated with zoledronic acid. The most common side effects overall were fatigue/asthenia and nausea, but the majority of patients were also treated with chemotherapy on these trials. While no deaths from hypocalcemia have been reported in any of the phase 3 trials, rare (7) deaths have been described in patients with metastatic disease and co-morbidities or low serum calcium levels prior to initiation of denosumab therapy. Patients with severe renal dysfunction (CrCl < 30mL/min) are more likely to develop hypocalcemia. Data from these registration trials suggest renal insufficiency, nonadherence with vitamin D and calcium supplementation, prostate cancer, and lung cancer are risk factors for the development of grade 3 or 4 hypocalcemia.

In this trial, those patients with insufficient blood levels of calcium and/or vitamin D at baseline 1 will receive supplemental calcium 800 mg and vitamin D (minimum 1000 IU daily). All patients will have adequate vitamin D levels (25 hydroxy vitamin D >20 ng/mL) as quantified by serum levels prior to starting on therapy, be instructed on the symptoms of hypocalcemia, and have serum calcium levels measured prior to each denosumab dose to protect against the development of significant hypocalcemia.

10.1 Osteonecrosis of the Jaw (ONJ)

In the primary treatment phases of the 3 pivotal phase 3 trials comparing denosumab to zoledronic acid for preventing SREs, ONJ was confirmed by an independent adjudication committee in 1.8% of patients receiving denosumab group and 1.3% of patients in the zoledronic acid group. When also including ONJ events occurring over an additional 4 months of blinded study in each trial, the incidence of confirmed ONJ was 2.2% in patients who received denosumab. The median time to ONJ was 14 months (range: 4 – 25). Risk factors for ONJ included tooth extraction (60%), and local tooth infection (48%) (20).
The incidence of ONJ was more recently updated in 2016. In this publication, the patient-year adjusted incidence of confirmed ONJ was 1.1% in patients receiving long-term monthly denosumab therapy for either metastatic prostate or breast cancer during the first year of treatment with increasing incidence rates observed with longer durations of therapy. When considering only those patients being treated for metastatic breast cancer, the adjusted patient-year risk of ONJ was 0.9% for the first year of use (21). As our study limits denosumab administration to only 6 monthly doses or roughly half the 12 doses prescribed in these trials, the risk for ONJ in this study can be reasonably expected to be lower than the observed 0.9% incidence (or less than 10 out of 10,000 patients).

10.2 Hypocalcemia

Denosumab can cause severe symptomatic hypocalcemia, and fatal cases have been reported from the postmarketing setting based on a recent review of cases. Hypocalcemia needs to be corrected prior to initiating denosumab. Calcium levels have to be monitored and all patients adequately supplemented with calcium and vitamin D. If hypocalcemia occurs, additional short-term calcium supplementation may be necessary.

10.3 Atypical femoral fracture

Atypical femoral fracture has been reported with denosumab. These fractures can occur anywhere in the femoral shaft from just below the lesser trochanter to above the supracondylar flare and are transverse or short oblique in orientation without evidence of comminution. Atypical femoral fractures most commonly occur with minimal or no trauma to the affected area. They may be bilateral and many patients report prodromal pain in the affected areas, usually presenting as dull, aching thigh pain, weeks to months before a complete fracture occurs. During denosumab treatment, patients should be advised to report new or unusual thigh, hip or groin pain. Patients presenting with such symptoms should be evaluated for an incomplete femoral fractures, and the contralateral femur should also be examined.

10.4 Multiple vertebral fractures

There is a risk of for multiple vertebral fractures following discontinuation of Xgeva (denosumab 120mg) in subjects participating in ongoing clinical trials. These fractures are not due to bone metastases. The events occurred in post-menopausal women with malignancies who had previous fractures (vertebral or non-vertebral) or who had known osteoporosis. Xgeva's effects on bone are known to be reversible and bone turnover increases after Xgeva is discontinued. Following discontinuation of Xgeva during study conduct or after study completion the Investigators should consider the individual subject's risk for fractures and the benefit:risk ratio of initiating anti-resorptive therapy in accordance with local guidelines for managing osteoporosis.

10.5 Hypersensitivity

Clinically significant hypersensitivity including anaphylaxis has been reported with use of denosumab. Reactions may include hypotension, dyspnea, upper airway edema, lip swelling, rash, pruritus, and urticarial. Clinically significant hypersensitivity has been identified as a contraindication for treatment with denosumab. If an anaphylactic or other clinically significant allergic reaction occurs, appropriate therapy will be initiated and denosumab therapy permanently discontinued.

10.6 Musculoskeletal pain

A safety assessment was performed on a cumulative review of clinical trial data and study serious adverse event and non-study adverse event reports of musculoskeletal pain. Based on this assessment, musculoskeletal pain, including severe cases, has been identified as an adverse drug reaction based on data from the post marketing setting.

11. INFORMATION ON STUDY PROCEDURES

During the study, participants will be asked to provide an optional breast biopsy at baseline (immediately prior to initiating denosumab therapy) and again at the end of study (EOS) visit, which will occur 1 month after receiving the 6th and last injection of denosumab therapy. Patients will undergo non-contrast, magnetic resonance imaging of the breast at baseline, and at the EOS visit as well. These procedures and their risks are described below.

11.1 Breast Biopsy Procedure

All participants will be asked to provide an optional core biopsy sample at baseline and after 6 months on denosumab; we estimate that ~75% will agree to the first and ~60% will agree to the second request, yielding approximately 12-13 pre- or postmenopausal participants with both baseline and 6-month on denosumab biopsies. For participants who consent to breast biopsy at baseline, the study subject will receive clinical orders to see a breast specialist (radiologist or surgeon) as a fee for service, and will use mammography images to identify two areas of increased radiographic density, located at least 2 cm apart, in the upper, outer quadrant of the contralateral (uninvolved) breast. For premenopausal women only, biopsies will be obtained during the luteal

phase of menses or days 18-24 after initiation of menses. Mammographically dense areas will be co-localized with ultrasound guidance, with their relative positions recorded by clock-face and linear distance away from the nipple. Under ultrasound guidance, a 14-gauge cutting needle will be used to obtain 8 tissue cores from one of the two identified high-density areas. Since 5-10 tissue cores are typically obtained during clinically indicated biopsy procedures, our tissue sampling protocol should not impose undue participant burden or risk.

11.2 Risk associated with Breast Biopsy

A breast biopsy procedure will be offered to all participants as an optional procedure. Potential risks associated with biopsy include: bleeding/bruising/hematoma formation at the biopsy site, pain at the site, rare instances of infection, and a small scar at the site. The risk of significant bleeding complications requiring surgery or hospitalization is nil. However, there is a 30% risk of hematoma or bruising after breast biopsy that spontaneously resolves.

11.3 Breast Imaging Procedure

All participants will undergo breast imaging by non-contrast MRI to obtain three-dimensional (3D) fat water ratio mapping at baseline (MRI_{BL}) and at the EOS (MRI_{EOS}) visit (6 months on study). All imaging procedures will adhere to FDA and OSHA guidelines. Images will be acquired on a Siemens 3T scanner with breast coils located at Stony Brook University Imaging Center. The MRI facility is shared by the hospital and research investigators.

11.4 FWR-MRI methods

Fat-water imaging will be performed on all patients. This method provides a robust lipid/water separation, which includes correction for field inhomogeneity and T2* decay. Data are acquired with various phase shifts between the fat and water signals. The separation of fat and water is then performed using an iterative algorithm (22). A 6-echo gradient echo sequence will be used.

11.5 Risk associated with Breast MRI

The Magnetic Resonance Imaging protocols utilized in this study use standard magnetic fields and do not involve contrast reagents. Thus, there are no known risks to the participants from the imaging protocols. However, patients with severe claustrophobia or electrically, magnetically, or mechanically activated implants (pacemakers, cochlear implants, etc.) are ineligible secondary to the increased risks from imaging inherent with their participation.

12. PARTICIPANT ELIGIBILITY

Eligibility criteria will include**:**

- Women with first incidence of early stage (stages 0 – III) breast cancer, including DCIS and LCIS, who have completed all treatment, with the exception of aromatase inhibitors, and are cancer-free. Patients who do not have *in situ* or invasive cancers, but solely high risk lesions or are at increased risk of breast cancer (e.g. breast biopsies showing atypical ductal or lobular hyperplasia (ADH, ALH), with or without atypia, and/or genetic risk (i.e. BRCA 1/2 mutations)) do NOT qualify for the study.
- Age ≥18 and ≤ 75 years
- 44 patients will be accrued as follows: either premenopausal as defined as regular menses (24-38 days) or postmenopausal/perimenopausal as defined by a history of amenorrhea for at least one year or hormone levels (estradiol/FSH) consistent with menopause if post-hysterectomy status, or history of surgical/medical castration, or having periods outside of the 24-38 days' range. We estimate a 10% drop out rate.
- Serum calcium or albumin-adjusted serum calcium must be ≥2.0mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL)
- Vitamin D level (25-hydroxy vitamin D level) must be > 20 ng/mL at baseline
- At least 1 month post-surgery, -radiation or -chemotherapy
- No prior or current use of IV bisphosphonates
- No current use of oral bisphosphonates
- Patients must have an unaffected, non-irradiated contralateral breast

- Significant breast density as determined by mammography and defined by the descriptive terms scattered fibroglandular tissue/densities, heterogeneously dense, or mostly dense tissue in the mammography report.
- Adequate renal function defined as a serum creatinine < 1.5 x ULN or CrCl ≥ 30mL/min
- A willingness and ability to follow the study protocol, as indicated by provision of informed consent to participate
- Willingness to being tested for current pregnancy and use of birth control while being treated with denosumab (pre-menopausal women only)

Exclusionary criteria will include:

- Age > 75 or < 18 years
- Subject has known sensitivity to any of the products to be administered during the study (e.g., mammalian derived products, denosumab, calcium, or vitamin D).
- Patients have prior history or current evidence of osteonecrosis or osteomyelitis of the jaw.
- Patients have active dental or jaw condition which requires oral surgery, including tooth extraction.
- Patients have non-healed dental or oral surgery, including tooth extraction.
- Patients with planned invasive dental procedures
- Subject is pregnant or breast feeding, or planning to become pregnant within 5 months after the end of the treatment
- Subject is of child bearing potential and is not willing to use, in combination with her partner, highly effective methods of contraception or abstinence during treatment and for 5 months after the end of treatment
- Active infection with Hepatitis B, Hepatitis C, or Human Immunodeficiency virus (HIV)
- Any condition or disorder that compromises the ability of the subject to provide written informed consent and/or comply with study procedures
- History of claustrophobia
- Have electrically, magnetically, or mechanically activated implants including cardiac pacemaker, cochlear implants, magnetic surgical clips or prostheses.
- Patients have/had thyroid/parathyroid surgery, malabsorption syndromes, hypoparathyroidism

13. STUDY PARTICIPANT IDENTIFICATION

Participants who have been consented will be identified on study-related documentation and forms with a unique 8 alphanumeric study identifier. These numbers will be issued to participants sequentially and no participant identification numbers will be re-assigned in the event that the participant is withdrawn from the study.

14. ASSIGNMENT TO STUDY GROUPS

The study will be a single institution, non-randomized trial. All the consented participants will be given the study intervention. Perimenopausal women will be considered as postmenopausal, for the goals of this trial. In case of significant menstrual period frequency changes during the course of the study (e.g. premenopausal participants becoming peri-menopausal, or those resuming a regular period at the end of their breast cancer treatment, but originally enrolled as peri-postmenopausal), will be required to sign the consent of the new corresponding study group and visits will be scheduled accordingly for the remaining of the study. The coordinator will document schedule changes in a nurse's note and an administrative tracking form.
Patients who underwent hysterectomy without or unilateral salpingo-oophorectomy (removal of ovaries) in the previous 12 months will be tested for levels of estradiol, follicule-stimulating (FSH) hormone at screening, in order to determine their menopausal status.

15. STUDY PLAN AND SCHEDULE OF EVENTS

The proposed study will be a non-randomized trial of pre- and postmenopausal women previously diagnosed with early stage breast cancer. Women will receive denosumab 120 mg by subcutaneous injection monthly for 6 months. We will attempt to recruit all eligible patients from the breast cancer clinics of the Stony Brook Cancer Center. (Appendix 1.1 and 1.2: Schedule of Events).

A total of 44 participants will receive denosumab 120 mg monthly for 6 months. All participants will undergo two MR imaging studies during the study. For the primary endpoints, participants will undergo MR Imaging at baseline and then again following 6 months on denosumab therapy. For premenopausal women, all MRI scans will be performed within 14 days from the beginning of their menses to ensure the scan is performed during the follicular phase of their menstrual cycle. Blood and urine will be obtained at baseline, and after 3 and 6 months on denosumab for measures of RANK ligand and OPG as well as for future studies of effects of denosumab on urinary hormone metabolites. Blood and urine will be obtained at the time of breast biopsy in all patients who consented to this procedure.

16. PARTICIPANT RECRUITMENT AND INFORMED CONSENT

Stony Brook Cancer Center staff not directly reporting to the PI Dr. Alison Stopeck will be responsible for identifying and recruiting candidate participants, evaluating their suitability for participation in the study, following participants during the treatment phase of the trial, monitoring participants for possible toxicity, and continuing to follow participants for subsequent toxicity during the post-treatment follow-up period.
Recruitment will proceed under the auspices of our Clinical Trials Office. Working in accordance with HIPAA regulations, study coordinators or treating medical staff who are not directly reporting to Dr. Alison Stopeck will identify potential participants and they will be screened for eligibility to the study. Recruitment will occur in the breast cancer and high risk clinics of Stony Brook University.

The research nurse will explain all aspects of the study in lay language to the potential eligible participants, answer all questions regarding the study, and confirm eligibility. If the participant decides to participate in the study, she will be asked to review, sign and date the Informed Consent Form (ICF). The ICF has to be completed prior to initiating any study related procedures. The study agent will not be released to a participant who has not signed the ICF. Participants may terminate or withdraw from the study at any time and for any reason, including development of an adverse event (AE) or serious adverse event (SAE), noncompliance, medical contraindication, or desire without prejudice.

17. SCREENING AND BASELINE VISIT PROCEDURES

All participants will be asked to provide an optional core biopsy sample at their baseline visit. For participants who consent to breast biopsy at baseline, the breast specialist at Stony Brook University (radiologist or surgeon), as a fee for service, will use mammography images to identify two areas of increased radiographic density, located at least 2 cm apart, in the upper, outer quadrant of the contralateral (uninvolved) breast. Mammographically dense areas will be co-localized with ultrasound guidance, with their relative positions recorded by clock-face and linear distance away from the nipple. Under ultrasound guidance, a 14-gauge cutting needle will be used to obtain up to 8 tissue cores from one of the two identified high-density areas. Since 5-10 tissue cores are typically obtained during clinically indicated biopsy procedures, our tissue sampling protocol should not impose undue participant burden or risk. Representative FFPE (formalin fixed and paraffin embedded) specimens will be reviewed at Stony Brook University by pathology under the direction of Dr. Ken Shroyer, a board-certified anatomical pathologist who specializes in breast pathology. All research tissue cores are processed at Stony Brook University. Evaluation of change in gene expression related to apoptotic, proliferation, and alternative biomarkers will be conducted in the laboratory of Dr. Thompson at Cedars Sinai Hospital, Los Angeles CA. All breast biopsies will be obtained 21 days +/- 3 days after the initiation of menses in premenopausal women to ensure that the biopsies are obtained during the luteal phase. A Material Transfer Agreement will be in place, covering shipping and receiving of study samples.

Blood chemistry results obtained up to 6 months prior to enrollment are considered valid for eligibility assessment. All other screening evaluations will be completed within 21 days of initiating the study.

The following events will occur at screening/baseline:

- Medical history including demographics (race, ethnicity, age, and etc.);
- Review concomitant medication and supplement use in the past 30 days;
- Vital signs (blood pressure, pulse);
- Height and weight;

- Hematology (CBC/differential and platelet count) (results obtained up to 6 months prior to study initiation are valid for eligibility);
- Chemistry panel (to include urea nitrogen, creatinine, ALT, AST, calcium, and phosphorus) (results obtained up to 6 months prior to study initiation are valid for eligibility);
- Serum 25-hydroxy vitamin D level (serum vitamin D levels should be at or above 20 ng/mL). Additional vitamin D testing is not required unless clinically indicated).
- Serum calcium level
- Quantification of estradiol, FSH and LH (for women who underwent hysterectomy within the previous 12 months and at least 1 intact ovary)
- Urine pregnancy test (pre-menopausal only) at each visit, ahead of treatment with denosumab

18. TREATMENT PLAN AND EVENTS

On study, injection visits will be scheduled every month (±10 days). The first 2 injections will be administered at Stony Brook Cancer Center, after ensuring that participants' blood calcium is within the accepted range (between 8.0mg/dL and 11.5 mg/dL). Upon patients' request, the remainder of denosumab injections (injections 3 through 6) will be administered at the participants' homes. End of study (EOS) procedures (e.g. biopsy, MRI, research blood and urine etc.) will be conducted at the Cancer Center approximately 1 month after the 6th, and last, injection. See Appendix 1.1 and 1.2 for a more detailed study plan.

The following events will occur on study.

- Denosumab 120 mg will be given subcutaneously at monthly intervals for a total of 6 injections
- A chemistry panel (to include serum 25-hydroxy vitamin D, urea nitrogen, creatinine, ALT, AST, calcium, and phosphorus) will be performed at baseline.
- Blood calcium levels will be checked before injections 1, 2, 3 and again approximately 8 weeks after the last denosumab injection. If serum calcium level is lower than 2.0 mmol/L (8.0 mg/dL) the participant will not receive the study drug and will be prescribed calcium supplements. If symptomatic, the patient will be evaluated by a provider, or if asymptomatic their provider will be notified. Once calcium levels are within range they may resume the study, but will be required to receive all study injections at the Cancer Center, and blood calcium levels will be measured and confirmed to be within range immediately prior to each injection.
- Blood for serum OPG and RANK ligand levels will be obtained at baseline and at EOS visit (in premenopausal women, blood will be obtained during the luteal phase or days 18-24 after initiation of menses) (research blood)
- A breast core biopsy at baseline and at the EOS visit for planned biomarker studies (OPG and RANK ligand) and storage for future studies (All breast biopsies will be obtained 21 days +/- 3 days after the initiation of menses in premenopausal women to ensure that the biopsies are obtained during the luteal phase. Urine and blood specimens will be obtained at the same time as breast biopsy for hormone levels).
- Two non-contrast breast MRIs will be obtained over the course of this study (in premenopausal women breast MRI scans will be performed within 14 days from the beginning of menses, during the follicular phase) at baseline and again ~1 month after the 6th denosumab injection
- A urine sample for research biomarker studies will be obtained at baseline and at the EOS visit (in premenopausal women, urine will be obtained during the luteal phase or days 18-24 after initiation of menses) (research urine)
- Vital signs (blood pressure, pulse);
- Height and weight;
- Review concomitant medications and supplements use in the past 30 days;
- A 10 days' window will be allowed for specimen collection and all procedures in postmenopausal women.
-

18.1 COVID-19 pandemic risk mitigations and exceptions

Due to circumstances linked to the COVID-19 pandemic, we are implementing the following procedures, aimed at maximizing the safety of study subjects and staff and adherent to current hospital policies:

- Subjects who have any self-reported or documented symptoms compatible with COVID-19 infection 24 hours prior to dosing or at the time of dosing, will not receive the study drug. Dosing will also be held at the discretion of the study investigator. Such situations will be documented, but will not be considered deviations.

- As currently specified in the protocol, subjects who miss two doses, for any reason, will be removed from the study and considered not evaluable.

- Subjects may elect to have their calcium tested at a lab of their choice ahead of dosing with study drug in in order to avoid coming to the Stony Brook Cancer Center.

- However, if subjects' insurance does not cover monthly calcium testing, subjects with normal, non-decreasing, calcium values may skip the third blood calcium testing (i.e. ahead of third denosumab injection visit) at the discretion of study investigators, in order to limit contacts. This still constitutes a deviation reportable to the IRB.

- In order to minimize contact between study staff and subjects at the time of home visits, body measurements and vitals will not be documented. This will not be considered a deviation and will not impact subjects' safety or data integrity.

- The final MRI/Biopsy may be postponed up to an additional 6 weeks based on COVID-19 precautions.

Subjects will be verbally notified of these changes and the communications will be documented in the subjects' binders.

19. DEFINITIONS

19.1 Follow-up:

All participants will have a follow-up visit or telephone contact scheduled two months after the last dose of study medication/end-of-treatment to follow-up for AEs and SAEs that may have occurred during or after discontinuation from the study. AEs and SAEs probably or possibly caused by the study drug that are still ongoing at the time of discontinuation will continue to be followed weekly until resolution or stabilization of condition. Similarly, abnormal laboratory values probably or possibly caused by the study drug will be followed every 14 days until resolution or two months post last dose of study drug(s) whichever occurs first.

19.2 End of Treatment:

Study drug discontinuation is the time when study agent(s) administration is permanently discontinued for any reason.

19.3 End of Study:

Defined as the time when a participant is permanently discontinued from any study evaluation for any reason.

19.4 Withdrawal of Participant from Study:

Participants will be withdrawn from the study under the following circumstances:

- Study closure,
- Unacceptable adverse event(s), as judged by the study physician,
- Participant decision to withdraw from the study or, in the judgment of the investigator, further participation would not be in the best interest of the participant,
- The participant is noncompliant with study procedures

19.5 Post-Intervention Evaluation and off-study monitoring:

An end of study visit (EOS) visit will be scheduled ~1 month after the last denosumab injection or at early termination, if applicable. Calcium will be tested 2 months after the last injection at a follow up visit.

20. AGENT ADMINISTRATION

Denosumab - Study agent (Denosumab injectable, 120 mg active) will be nurse-administered. Toxicities associated with denosumab administration include pain at the injection site and symptoms of drug hypersensitivity including headache, rash, flushing, swelling, shortness of breath, nausea, or vomiting. Clinically significant hypersensitivity including anaphylaxis has been reported with use of denosumab. Reactions may include hypotension, dyspnea, upper airway edema, lip swelling, rash, pruritus, and urticaria.

21. CONCOMITANT MEDICATIONS

Patients may be instructed to take daily supplements of at least 800 mg calcium and at least 1000 IU of vitamin D, unless contraindicated or documented hypercalcemia (albumin-adjusted serum calcium >2.9mmol/L [11.5mg/dL] or ionized calcium >1.5mmol/L [6.0mg/dL] develops on study.
Due to differences in regional availability, a dosage form of Vitamin D that gives an equivalent of at least 1000 IU daily may be given.
Patients on therapy with aromatase inhibitors will continue the treatment as clinically indicated. Changes in agent, dose or frequency will be captured in a Concomitant Medications Form, and reviewed at each study visit, and during the Follow Up phone call.

22. TOXICITY MONITORING AND RESPONSE PLAN

- Administration of investigational product (denosumab) will be withheld for any subject who experiences a grade 3 or 4 adverse event per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) reported by the investigator as related to investigational product, or osteonecrosis of the jaw (ONJ) as determined by the investigator or by an independent expert panel. Treatment may also be withheld upon discretion of the study doctor for any reason to remove risk or minimized discomfort to participants. Re-exposure to investigational product may occur when the event resolves to grade 1 or less or the subject's baseline, and if the investigator and the sponsor agree subject safety will not be compromised.

- Osteonecrosis of the jaw (ONJ) can occur in subjects receiving denosumab. A dental examination with appropriate preventive dentistry should be considered prior to treatment with denosumab in patients with risk factors for ONJ. Patients who are suspected of having or who develop ONJ while on denosumab should receive care by a dentist or oral surgeon. Patients should be monitored for ONJ as part of routine clinical practice. Patients should be instructed to maintain good oral hygiene and avoid invasive dental procedures, if possible. Administration of investigational product (denosumab) will also be withheld 30 days prior to any elective invasive oral/dental procedure. Investigational product administration will be withheld until documented evidence of complete mucosal healing following any invasive oral/dental procedure.

- Participants that miss >2 doses of scheduled denosumab, regardless of cause, will discontinue study agent and proceed with end of treatment procedures.

- There will be no dose modifications for toxicity during this study.

23. END OF TREATMENT PROCEDURES

- Vital signs (Blood pressure, pulse);
- Blood and urine samples for banking (research)
- Review concomitant medications;
- Review adverse events;
- End of Treatment procedures will be performed one month after the last study agent administration.

- Following discontinuation of study agent participants experiencing adverse events will be contacted weekly by telephone or clinic visit until resolution or stabilization of condition. Abnormal laboratory values will be followed every 14 days until resolution or two months post last dose of study agent(s) whichever occurs first.

24. URINE SAMPLE COLLECTION, PROCESSING AND ANALYSES

Urine will be collected at baseline visit, and at the end of study visit. Urine may be also collected at an intermediate visit (injection 4) if the participant elects to visit the clinic to receive her study drug. All urine samples will be aliquoted in clinic the same day as collection and transported to the Thompson laboratory at Stony Brook Medical Center within 2 hours for storage at -80^{o}C for future analyses. Following the relocation of Dr. Thompson's laboratory to Cedars Sinai Hospital, Los Angeles, beginning January 2021, all previously and any newly collected urine samples will be transported to the Stony Brook Cancer Center Biobank where they will be stored at -80^{o}C for future research. All urine specimens are labeled and tracked with a unique sample ID. The Thompson research lab developed and deployed Standard Operating Procedures that follow all regulations pertaining to lab safety, sample processing, specimen classification and repository management. These SOPs will be transferred to the Cancer Center Biobank under the direction of Dr. Richard Kew beginning January 2021. Subsequent urine biomarker studies conducted in the Thompson laboratory at Cedars Sinai Hospital will be conducted under a Material Transfer Agreement covering shipping, receiving and allocation of study samples.

25. BLOOD SAMPLE COLLECTION, PROCESSING AND ANALYSES

25.1 Clinical Blood Chemistry and Calcium Monitoring

Blood for full clinical blood chemistry will be collected when enrollment is initiated following consent, at baseline. Before injections 1, 2, 3 and approximately 8 weeks after the last denosumab injection blood will be drawn to check calcium level. The chemistry panel, blood calcium, and pregnancy test are performed by a fee for service laboratory routinely utilized by the Cancer Center. The service lab holds current accreditation issued by the College of American Pathologists' Laboratory Accreditation Program.

25.2 Blood Banking

Blood will be collected at baseline visit and EOS visit. Blood for research purposes may be also collected at an intermediate visit (injection 4) if the participant elects to visit the clinic to receive her study drug. All blood samples will be aliquoted in clinic the same day as collection and transported to the Thompson laboratory at Stony Brook Medical Center within 2 hours for storage at -80^{o}C for future analyses. Whole blood will be collected only once during the study at the first Baseline visit. Serum for banking will be collected in a Red top Vacutainer. Plasma for banking will be collected in a Lavender top EDTA Vacutainer. Whole blood will be collected from the same Lavender top EDTA Vacutainer. Vacutainer tubes are centrifuged the same day as collection. All blood specimens are labeled and tracked with a unique sample ID. All blood samples will be processed to serum and plasma and transported within 2 hours for storage in aliquots at -80°C for future analysis. All blood derived specimens will be stored in the Cancer Center Biobank. Following the relocation of Dr. Thompson's laboratory to Cedars Sinai Hospital, Los Angeles, beginning January 2021, all previously and any newly collected blood sample products will be transported to the Stony Brook Cancer Center Biobank where they will be stored at -80^{o}C for future research. All urine specimens are labeled and tracked with a unique sample ID. The Thompson research lab developed and deployed Standard Operating Procedures that follow all regulations pertaining to lab safety, sample processing, specimen classification and repository management. These SOPs will be transferred to the Cancer Center Biobank under the direction of Dr. Richard Kew beginning January 2021. Subsequent future blood based biomarker studies conducted in the Thompson laboratory at Cedars Sinai Hospital will be conducted under a Material Transfer Agreement covering shipping, receiving and allocation of study samples.

26. DRUG FORMULATION AND PROCUREMENT

26.1 Denosumab Physical and Chemical Characteristics

Denosumab is a fully human monoclonal IgG2 antibody that binds with high affinity and specificity to RANK ligand (Kd 3 x 10-12 M). This binding prevents the activation of RANK and inhibits the formation, activation, and survival of osteoclasts, the result of which is a reduction in the number and function of osteoclasts and, consequently, a decrease in bone resorption and an increase in cortical and trabecular bone mass, volume, and strength. Denosumab is highly specific because it binds only to RANK ligand and does not bind to other members of the tumor necrosis factor (TNF) family, including TNFα, TNFβ, TNF-related apoptosis-inducing ligand, or CD40 ligand. Denosumab has an approximate molecular weight of 147kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells. Denosumab will be supplied as a sterile, clear, colorless to slightly yellow, preservative-free liquid, in single-use 3.0 mL glass vials containing a deliverable dose of 1.7 mL. .

26.2 Availability

Denosumab is available as 120mg/1.7mL in single-use vials and will be obtained from the Stony Brook Cancer Center Pharmacy and supplied by Amgen. Study agent will be stored in the Stony Brook Cancer Center Pharmacy. Denosumab is a clear, colorless to pale yellow solution that may contain trace amounts of translucent to white proteinaceous particles. Prior to administration, denosumab will be removed from the refrigerator and brought to room temperature by standing in the original container. A 27-gauge needle will be used to withdraw and inject the entire contents of the vial subcutaneously into the upper arm, upper thigh, or abdomen of the subjects.

26.3 Agent Accountability

Stony Brook Cancer Center Pharmacy will maintain a careful record of the inventory and disposition of all agents received using the NCI Drug Accountability Record Form (DARF) or similar record. Duties include maintaining adequate records of receipt, dispensing and final disposition of study agent(s).

26.4 Packaging and Labels

Denosumab will be provided by Amgen and dispensed through the Stony Brook Cancer Center by a licensed oncology pharmacist and dispensed by the treating physician's order.

26.5 Storage

Study medication will be stored at room temperature (59° to 86° F), protected from environmental extremes and in a locked cabinet or room prior to being issued to participants.

27. STATISTICAL CONSIDERATIONS

27.1 Statistical analysis plan

The primary endpoint of the study is the change in breast density of the contralateral, uninvolved breast as measured by quantitative Fat Water Ratio (FWR-MRI) mapping after 6 months on denosumab compared to baseline. Statistical analysis for the primary endpoint will estimate the change in breast density between 0 and 6 months, which is defined as (baseline BD-month 6 BD)/baseline BD. Percentages of patients who have at least 5% drop in BD and corresponding 90% Blyth-Casella-Still confidence intervals (CI) will be reported.

Analysis of the exploratory endpoints of changes in tissue composition, gene expression patterns, hormone levels and OPG/RANKL ratio will be performed using paired t-tests or Wilcoxon signed rank tests if the data are not normally distributed. Correlations between hormone levels and OPG/RANKL ratio with the FWR-MRI acquired MRI-derived BD values at baseline and 6 months after therapy will be estimated using linear mixed effect models for repeated measures where patient will be treated as a random effect. This model also allows to test if such correlation changes after intervention. Model assumption will be diagnosed and data transformation may be necessary to make the assumption met.

27.2 Power Estimation

In this pilot study we assume an initial sample size of 44 either pre-menopausal or post-menopausal women and allow the possibility of ~10% dropout for the 6-month measurement. This results in about 40 evaluable women. The primary trial endpoint is defined as the difference in the change in FWR-MRI-derived BD after 6 months on

intervention. Our study hypothesis is that at least 30% patients in each group will have ≥5% drop in BD after 6-month Denosumab therapy. Table 1 below shows the estimated rate of patients who had ≥5% drop and its corresponding 95% Blyth-Casella-Still CIs using 40 evaluable patients. Table 1 suggests that if there are 18 out of 40 patients having ≥5% drop in BD, we are 90% confident to conclude that at least 31.7% patients will have ≥5% drop in BD after 6 months of Denosumab therapy.

Table 1: Estimated rates of patients who have ≥5% drop in BD and corresponding 90% Blyth-Casella-Still confidence intervals based on 40 evaluable patients

# of patients having ≥5% drop in BD after 6 months of Denosumab therapy	Estimated rate	90% Blyth-Casella-Still CI
12	30%	18.5 – 43.3%
14	35%	23.4 – 48.3%
16	40%	26.9 – 54.2%
18	45%	31.7 – 59.1%
19	47.5%	35 – 61.5%

28. ADMINISTRATIVE CONSIDERATIONS

28.1 Regulatory Board Review

The study protocol will be activated after review and approval by the Stony Brook Cancer Center Protocol Review Committee and the University's Human Subjects Protection Program (IRB). Approval of this protocol by the IRB will also include approval of the protocol consenting document, or Informed Consent Form (ICF) and the accompanying HIPAA consenting instrument.

28.2 Compliance with Protocol and Protocol Revisions

Study PI and CO-PI will conduct the study as described in this protocol. Any protocol revisions made in amendments to the protocol by the Principal Investigator will be approved by the IRB prior to implementation, except where necessary to avoid imminent hazard to a study participant. If an amendment alters the study design or increases the potential risk to the participant, the ICF will be revised and submitted for approval to the IRB. The revised ICF will be used to obtain consent from participants currently enrolled in the study or after withdrawal from the study if they are affected by the Amendment.

28.3 Participant Informed Consent

All study participants will sign the most current ICF that has been approved by the IRB prior to enrollment on the study. Investigators will have ensured that participants are clearly and fully informed about the purpose, potential risks and any other critical issues regarding this clinical trial.

28.4 Data Management and Safety Monitoring

Participant safety and data integrity will be monitored by the Stony Brook Cancer Center's Data Safety and Monitoring Committee (DSMC) in conjunction with the Quality Assurance/Quality Control Program (QA/QC). The Protocol Review Committee approval letter, which states the frequency of data review, is attached in Appendix 8.

Prior to study registration, a review of the potential participant's eligibility will include a confirmation of the inclusion/exclusion criteria and proper execution of informed consent. All participants registered to this study will be entered into the Stony Brook Cancer Center database for accrual and treatment status tracking. In addition, a registration log will be maintained utilizing the pre-prepared form to keep track of study number, verification of consent, date of consent, and other relevant registration data. Electronic Case Report Forms (CRFs) will be utilized. All participant study files will be stored in a secure area limited to authorized staff.

The Principal Investigator will ensure the accuracy, completeness and timeliness of the data reported in these forms. Source documentation supporting the CRF data should indicate the participant's participation in the trial and should document the dates and details of study procedures, adverse events, and participant status.

The Stony Brook study team will generate a coded database and remove PHIs (the code will remain at SB) and send it via secure email communication to investigators at Cedars-Sinai.

29. DATA SAFETY AND MONITORING PLAN

29.1 Protocol Data and Safety Monitoring Plan and Risk Level Designation.

29.1.1 Risk Level.

This trial has been designated as a medium risk study. Medium risk studies are intended to include all trials involving therapeutic intervention(s), which are not designated as high risk per NCI and the IND is not held by the investigator.

29.1.2 Identification of the DSMC obligated for oversight responsibilities:

The Stony Brook Cancer Center Data Safety and Monitoring Committee (DSMC) will provide ongoing oversight for this trial semi-annually. In addition, the investigators will convene an ad hoc DSMC comprised of Alexander Stessin MD, PhD, a radiation oncologist with expertise in breast cancer, a statistician (Erin Taub), and will be chaired by Marie Gelato, MD, PhD, an endocrinologist with expertise in osteoporosis and hypocalcemia. The *ad hoc* DSMC will initially convene after 10% of the projected accrual, or 4 patients, complete protocol therapy, and thereafter annually. While no formal stopping rules are included in the protocol, accrual to the study will be suspended if 2 or more patients experience grade 3 or higher hypocalcemia or ONJ events. In the unlikely event that this occurs, the DSMC will be convened to review all safety data and make recommendations for either continuing the study with additional safety measures or terminating the study altogether. A dental expert will be added to the committee if 2 or more potential ONJ events are observed.

29.1.3 Identification of the entity obligated for routine monitoring duties

Routine monitoring will be provided by the Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements.

29.1.4 Monitoring progress and data review process

Routine monitoring of participant data will be conducted at least every 6 months.

The first routine monitoring visit will include at a minimum

- Informed consent cases enrolled, according to PRMC policy;
- Participant eligibility, up to two participants, according to PRMC policy;
- Data review, up to two participants, according to PRMC policy.

All subsequent monitoring visits will consist of randomly selected participant cases based on current enrollment and include continuing review of previously selected cases, as applicable. A monitoring visit report and follow-up letter will be completed within two weeks of the routine monitoring visit; a copy will be maintained in the study file. A query/finding form will also be completed by the monitor to request additional source documentation, clarification, information or corrections to the CRF and/or regulatory records. The Clinical Research Coordinator or other applicable staff responsible for the study will be given a copy of this form for resolution of queries/findings. The query/finding form will be maintained with a copy of the visit report for follow-up at the next monitoring visit.

The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF). Source documentation supporting the CRF data should indicate the participant's participation in the trial and should document the dates and details of study procedures, adverse events, and participant status. Case report forms, which include the inclusion/exclusion criteria form, adverse event forms and serious adverse event forms should be completed with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All participant forms and study files will be stored in a secure area limited to authorized staff. *Note:* Routine monitoring of regulatory documents and test article will be conducted at least annually. A process to implement study closure when significant risks or benefits is in place.

30. DESCRIPTION AND REPORTING OF ADVERSE AND SERIOUS ADVERSE EVENTS

Serious adverse events (SAEs) and adverse events (AEs) will be monitored and discussed routinely using procedures described below. Cumulative SAE review is performed by the SAE Initial Trend Analysis Team monthly. This review not only identifies individual events but also identifies any patterns of adverse events in Investigator initiated trials. If there is concern for excessive risk/toxicity identified by the DSMB, the Committee may recommend suspension of accrual or study closure.
All SAEs (Death, A life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect.) must be reported to Amgen Drug Safety within 24 hours of discovery or notification of the event. Initial SAE information and all amendments or additions must be recorded on an SAE Report form and faxed to Amgen at: Amgen Global Safety Fax: 888-814-8653.

30.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Any adverse events grade 2, or greater, will be recorded on the adverse events record form and reviewed by the Principal Investigator.

Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5
(https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)
And will address:

- Grade
- Relationship to study drug(not related, unlikely, possible, probable, definitely)
- Causality other than study drug (disease related, concomitant medication related, intercurrent illness, other)
- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, held, dose reduced, discontinued, medication given)

30.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening;
- A new cancer
- Requires in-patient hospitalization or prolongation of an existing hospital stay;
- Results in persistent disability or significant disability/incapacity, or:
- Is a congenital anomaly/birth defect.

Note: A SAE may also be an important medical event, in the view of the investigator that requires medical or surgical intervention to prevent one of the outcomes listed above.
Serious adverse events will be captured beginning on Day 1 when participants consent to the study and continuing one month following the last dose of study agent.

All serious adverse events, regardless of attribution, and any deaths will be reported within 24 hours of notification of the event to the DSMB Coordinator and to Amgen. All SAEs which meet the criteria for a reportable

event will be reported to Stony Brook University IRB within 10 working days of the event date or receipt of notification of the event.

30.3 Reportable events

Reportable events must meet all three of the following criteria:

- Unanticipated or unexpected in nature, frequency or severity, AND
- Related or possibly related to participation in the research (there is a reasonable possibility that it is related), AND
- The event suggests that the research places participants or others at a greater risk of harm than was previously known or recognized (not already referenced in existing study documents, such as the protocol, consent form or Investigator's Brochure).

All serious adverse events will be processed by the DSMB Coordinator monthly for initial trend analysis and fully reviewed by the DSMB, every six months. The DSMB coordinator will review the SAE reporting process to confirm reporting requirements are met.

30.4 Plan for assuring data accuracy and protocol compliance

Routine study activity and safety information will be reported to the DSMB every six months, or more frequently if requested. These reports will include:

- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies.
- Data, safety and study progress will be reported to:
  Human Subjects Protection Program (IRB) at least annually;
  SAEs will be reported to the Sponsor (Amgen) every 90 days.
- Pregnancy: The investigators must report pregnancy of a subject participating in the clinical trial to Amgen's global Pregnancy Surveillance Program within 10 days of receiving notification of a pregnancy. Follow up case reports have to be provided as new information becomes available. The Amgen Pharmacovigilance Program will seek to follow the pregnant woman throughout her pregnancy and her baby up to 12 months after the birth.
- The PI will immediately notify, in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.

30.5 Additional Adverse Events Reporting

All SAEs which meet the criteria for a reportable event will be reported to the Stony Brook University IRB and to the Sponsor within 10 working days of the event date or receipt of notification of the event.

31. PARTICIPANT PAYMENT

Biopsies will be compensated with $100, and MRIs with $25, for a maximum total of $250 for participants undergoing MRI and biopsies, or $100 for MRI-only participants. Participants will not receive more than $100 per visit, i.e. when an MRI and a biopsy are done on the same day. When biopsies and MRIs are scheduled on different days, participants can receive 2 separate payments of $100 and $25 respectively.

32. APPENDIX 1.1 and 1.2: Study Schedule for pre-menopausal and post-menopausal women

33. APPENDIX 2: Reprints

34. APPENDIX 3: Common Toxicity Criteria, Version 5.0

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

35. APPENDIX 4: ECOG Performance Scale

http://www.npcrc.org/files/news/ECOG_performance_status.pdf

36. APPENDIX 5: Abbreviations

37. APPENDIX 6: Xgeva® package insert

38. APPENDIX 7: Protocol Review Committee Approval letter

39. APPENDIX 8: Stony Brook Cancer Center PRMC policy

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