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Pilot study of denosumab in *BRCA1/2* mutation carriers scheduling risk-reducing salpingo-oophorectomy

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SCHEMA

Pilot study of denosumab in *BRCA1/2* mutation carriers scheduling risk-reducing salpingo-oophorectomy

Premenopausal *BRCA1/2* mutation carriers scheduling risk-reducing salpingo-oophorectomy, with or without hysterectomy (N=60)

↓

Baseline data collection (within 30 days prior to randomization)
Informed consent, registration, physical exam, concomitant medications, baseline symptoms, pregnancy test (within 14 days prior to randomization), blood draw for clinical lab tests, circulating biomarkers, and biorepository

↓

Randomization
Participants will be stratified by *BRCA1*/*BRCA2* mutation status and hormonal contraceptive use

1-2 doses of Denosumab 120 mg SQ q4wks Calcium 1000 mg and Vitamin D3 1000 IU daily x 6 months 30 randomized/27 evaluable	No denosumab treatment Calcium 1000 mg and Vitamin D3 1000 IU daily x 6 months 30 randomized/27 evaluable

↓

Intervention:
Denosumab 120 mg administered SQ Dose # 1 (Within the first 3 days of the menstrual cycle)

↓

Toxicity assessment and Calcium/Vitamin D compliance by telephone contact (Day 8-10 of menstrual cycle)

↓

Possible dose #2 of Denosumab 120 mg administered SQ 4 weeks (+/- 3 days) after dose #1 if needed (a dose is required within four weeks of surgery and does not need to be timed with menstrual cycle), blood draw (for clinical labs)

↓

Calcium/Vitamin D compliance q1month (+/- 1 week) x 6 months

↓

Surgery (Day 14-28 of menstrual cycle after last dose of denosumab **in treatment arm** or Day 14-28 of menstrual cycle within 2-8 weeks after initiating vitamin D/calcium supplement in **no treatment arm**), physical exam/vitals, concomitant medications, toxicity assessment, Calcium/Vitamin D compliance, blood draw (for circulating biomarkers and clinical labs), and tissue collection

↓

Calcium/Vitamin D compliance by telephone contact 1 week (+/- 7 days) post-surgery

↓

6 and 12 months (+/- 90 days) visits for toxicity and compliance, research blood draw for C-terminal telopeptide (to assess bone turnover after surgical menopause and stopping denosumab) and collection of pill bottles at 6-month visit

Primary endpoint: Ki67 proliferation index by immunohistochemistry (IHC) in fallopian tube fimbrial epithelial cells after denosumab treatment compared to no treatment

Secondary endpoints:

a) Ki67 proliferation index (IHC) in ovarian surface epithelium and endometrium (if also undergoing hysterectomy, ~20% of participants) after denosumab treatment compared to no treatment

b) Tissue-based biomarkers in the fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium (if also undergoing hysterectomy) after denosumab treatment compared to no treatment, including: apoptosis with

cleaved caspase-3 (IHC), RANK/RANKL (IHC), estrogen receptor (ER)/progesterone receptor (PR) (IHC), CD44 (IHC), p53 (IHC), STAT3 (IHC), and pSTAT3 (IHC)

c) Gene expression profiling of the fimbrial end of the fallopian tube and ovarian surface epithelium after denosumab treatment compared to no treatment
d) Change in serum biomarkers from baseline (pre-treatment) to time of surgery (post-treatment) with denosumab treatment compared to no treatment, including: progesterone, estradiol, and denosumab drug levels
e) Change in serial serum C-terminal telopeptide (CTX) from baseline (pre-treatment) to time of surgery to 6 months and 12 months after start of intervention with denosumab treatment compared to no treatment
f) Toxicity profile and frequency of adverse effects of denosumab treatment compared to no treatment

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1. OBJECTIVES

1.1 Primary Objectives

The primary objective of this study is to compare the effect of denosumab 120 mg subcutaneously every 4 weeks for 1-2 doses to no treatment in the pre-surgical setting on Ki67 proliferation index by immunohistochemistry (IHC) in the fimbrial end of the fallopian tube of premenopausal *BRCA1/2* mutation carriers undergoing risk-reducing salpingo-oophorectomy, with or without hysterectomy.

1.2 Secondary Objectives

The secondary objectives are:

- To assess Ki67 proliferation index by IHC in ovarian surface epithelium and endometrium (if also undergoing a hysterectomy, ~20% of participants) after exposure to denosumab compared to no treatment
- To investigate other tissue-based biomarkers in the fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium (if also undergoing hysterectomy) after exposure to denosumab compared to no treatment, including:
  - Apoptosis with cleaved caspase-3 by IHC
  - Receptor activator of NF-KB (RANK) and RANK ligand (RANKL) expression by IHC
  - Estrogen receptor (ER) and progesterone receptor (PR) expression by IHC
  - CD44 and p53 expression by IHC
  - Signal transducer and activator of transcription 3 (STAT3) and phosphorylated-STAT3 (pSTAT3) expression by IHC
- To analyze gene expression profiling in the fimbrial end of the fallopian tube and ovarian surface epithelium after exposure to denosumab compared to no treatment
- To investigate serum biomarkers at baseline (pre-treatment) and time of surgery (post-treatment) with denosumab compared to no treatment, including:
  - Progesterone
  - Estradiol
  - Denosumab drug levels
- To investigate serial serum C-terminal telopeptide (CTX) levels from baseline (pre-treatment) to time of surgery to 6 months and 12 months after start of intervention with denosumab compared to no treatment
- To monitor safety and adverse effects of denosumab compared to no treatment

2. BACKGROUND

2.1 *BRCA1/2* mutation carriers

Cancer risk and prevention in BRCA1/2 mutation carriers

BRCA1/2 genes play a role in DNA repair. The prevalence of *BRCA* mutations is 1 in 400 in the general population, whereas the prevalence of a founder mutation in the *BRCA1* (5382insC or 185delAG) or *BRCA2* (6174delT) genes is up to 2.5% (1 in 40) among individuals of Ashkenazi (central and eastern European) Jewish descent.[1,2] Female carriers of *BRCA1/2* mutations have an elevated lifetime risk of developing breast cancer and ovarian cancer of 40-60% and 20-40%, respectively.[1,3-5] High-grade serous carcinoma (HGSC) is the most common and fatal ovarian cancer histologic subtype and *BRCA1/2* mutations are found in 17% of HGSC cases.[6] Risk management options for mutation carriers include intensive breast cancer screening with clinical breast exam, mammography, and breast MRI, risk-reducing surgeries such as prophylactic mastectomy and bilateral salpingo-oophorectomy (BSO), and chemoprevention.[7-11] Unfortunately, there is no effective method of screening for ovarian cancer.[12,13]

With regards to risk-reducing surgery for ovarian cancer, the National Comprehensive Cancer Network (NCCN) recommends risk-reducing bilateral salpingo-oophorectomy (RR-BSO) between the ages of 35-40 or upon completion of childbearing.[12] RR-BSO was associated with a 72-86% relative risk reduction of ovarian cancer, 37% reduction in risk of breast cancer in *BRCA1* carriers, and a 64% reduction in risk of breast cancer in *BRCA2* carriers.[9] Mutation carriers who underwent RR-BSO compared to those who did not had a 79% reduction in ovarian cancer-specific mortality, 56% reduction in breast cancer-specific mortality[9], and a 60-77% reduction in all-cause mortality.[9,10] The prevalence of occult ovarian and fallopian tube cancers detected on RR-BSO in *BRCA* mutation carriers (excluding serous tubal intraepithelial carcinoma) has been reported between 1.8-17%.[14-20] While RR-BSO is effective in reducing risk of breast and ovarian cancer, it is also associated with adverse effects related to premature menopause, including osteoporosis, cardiovascular disease, cognitive impairment, and increased anxiety.[21] There is also data to suggest that *BRCA1* mutation carriers may have an increased risk for serous/serous-like endometrial carcinoma and thus the risks and benefits of undergoing hysterectomy at the time of RR-BSO should be discussed with the patient.[22]

Role of sex hormones in breast and ovarian cancer in BRCA1/2 carriers

While *BRCA1/2* mutations are believed to cause cancer via defects in DNA damage repair pathways, this does not explain the breast and ovary-specific cancer penetrance. Widschwendter *et al.* reported that *BRCA1/2* carriers have higher titers of serum estrogen and progesterone during the luteal phase of the menstrual cycle (**Figure 1**) and hypothesized that this sex hormone dysregulation and altered end-organ hormone sensitivity might explain the organ-specific penetrance.[23] While estrogen exposure is thought to be a risk factor for ovarian cancer, progesterone exposure has long been hypothesized to play a protective role in ovarian cancer.[24] This hypothesis is supported by numerous epidemiologic studies that have demonstrated a protective effect of pregnancy and oral contraceptive use on ovarian cancer risk, both of which increase levels of circulating progesterone. Premenopausal women with >10 years of oral contraceptive use had a significant decreased risk (odds ratio [OR]=0.3, 95% confidence interval [CI]=0.2-0.6), with reductions of 20-30% for each 5 years of use.[25] This protection is present with as little as 1-5 years of use (OR=0.62, 95% CI=0.56-0.69).[26] With regards to pregnancy, parous women were found to be at reduced risk of ovarian cancer compared with non-parous women (OR=0.5, 95% CI=0.3-0.7)[25], with protection increasing as number of pregnancies increases.[26] Studies in premenopausal *BRCA1/2* mutation carriers by the Hereditary Breast Cancer Clinical Study Group demonstrated a similar protective effect of oral contraceptive use and pregnancy on ovarian cancer risk.[27] While the protective role of progesterone has been well established, the mechanism by which it renders protection is still unclear. Experimental studies in cell lines and animals have demonstrated that progesterone exposure induces apoptosis through increased expression of p53 in ovarian epithelium[28-30]; however, additional studies are necessary to elucidate the mechanism by which progesterone influences proliferation and apoptosis in the ovaries of premenopausal women. Tubal ligation has also been associated with a reduction in the risk of ovarian cancer, with a meta-analysis of 13 studies showing a reduction in risk of epithelial ovarian cancer of 34% (risk ratio [RR] = 0.66, 95% CI=-.60-0.73).[31-33]

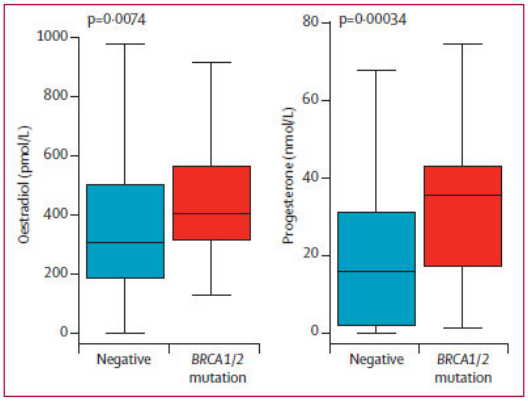

Figure 1. Serum estradiol and progesterone levels in *BRCA1/2* mutation carriers and non-carriers[23]

In contrast to its protective role in ovarian cancer, progesterone levels have been demonstrated to be associated with an increased risk of breast cancer. Epidemiologic studies have shown that postmenopausal estrogen and progesterone combined therapy increases proliferation in breast tissue, mammographic density, and breast cancer risk over estrogen alone.[34,35] In premenopausal women, oral contraceptive use as

well as high progesterone levels during pregnancy are associated with an increased risk of breast cancer.[36] In some animal models, tumor formation has been shown to be progesterone-dependent, with progesterone thought to play a role in the expansion of mammary stem cells.[37] This stem cell effect of progesterone is protected in part by the BRCA1 protein; thus, progesterone inhibitors or antagonists are being tested in mouse models of *BRCA1/2* mutations and among female carriers that are at high risk of developing breast cancer.

2.2 Rationale for Denosumab

Role of Receptor activator of NF-KB ligand (RANKL) signaling in BRCA1 tumorigenesis

RANKL is an osteoclast differentiation factor that is essential for the development and activation of osteoclasts. RANKL is secreted by progesterone receptor (PR)-positive epithelial cells in response to progesterone, and acts as a paracrine factor on the estrogen receptor (ER)/PR-negative progenitor cells through its receptor RANK (**Figure 2**). RANK is expressed in the luminal progenitor subset of cells and there is significantly increased RANK expression in progenitors from *BRCA1* mutation carriers when compared to *BRCA1* WT controls.[38] RANK and RANKL are expressed in breast tumors. In mouse models, RANK signaling have been shown to promote mammary tumor formation and progression,[39,40] while inhibition of RANKL signaling reduces mammary tumorigenesis.[41] RANK+ luminal progenitors are the probable 'cell-of-origin' for basal-like breast cancers.[38]

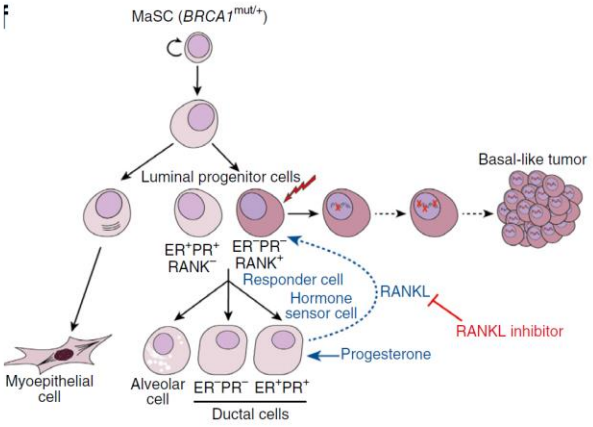

Figure 2. Role of RANKL in BRCA1 mammary tumorigenesis[38]

Denosumab

Denosumab is a monoclonal antibody that inhibits RANKL. It is approved for prevention of fractures in patients with osteoporosis (60 mg subcutaneously every 6 months) and in patients with bone metastases from solid tumor malignancies (120 mg subcutaneously every 4 weeks).[42-44] Denosumab has a good safety profile and the adverse effects include hypocalcemia, osteonecrosis of the jaw, atypical femoral fracture, and skin problems. The rate of serious adverse events in three randomized controlled trials over 4 years was 11% with placebo versus 18% with denosumab. There is limited data on adverse events in the setting of denosumab treatment discontinuation. One study investigated the adverse event rates over 24 months after discontinuation of denosumab 60 mg every 6 months for 24 months or placebo injections for 24 months in postmenopausal women with osteoporosis and found no difference in adverse event rates during the off-treatment phase between the 2 groups.[45] However, there have been case reports of rebound-associated vertebral fractures after discontinuation of denosumab in postmenopausal women with osteoporosis.[46]

Denosumab for Breast Cancer Prevention

Due to the link between progesterone signaling in breast cancer and RANKL contribution to mammary tumorigenesis, denosumab is being pursued as a potential preventive agent for breast cancer in *BRCA1/2* mutation carriers. Sigl *et al.* published a study of RANK signaling inhibition in two different mouse models of breast cancer.[47] In a *BRCA1^{mut} p53^{mut}* model, inactivation of RANK in the mammary epithelium resulted in delayed onset and reduced incidence and progression of mammary tumors. In addition, pharmacologic inhibition of RANKL eliminated the development of pre-neoplastic lesions. A study by Nolan *et al.* identified RANK+ luminal progenitor cells as putative cells of origin in *BRCA1* mutation carriers. Further, in an ongoing pre-surgical window of opportunity study in *BRCA1/2* mutation carriers, the investigators demonstrated that treatment with 120 mg denosumab monthly for 3 months resulted in decreased

proliferation in breast tissue as measured by Ki-67 staining.[38]

While these studies show promise for RANKL inhibition as a breast cancer prevention strategy in *BRCA1/2* mutation carriers, it is unclear whether the protective role of progesterone in ovarian cancer may be usurped by RANKL inhibition in this high-risk population. There is currently no published data on the effect of RANKL inhibition on gynecologic tissues, including fimbrial tissue, ovarian surface epithelium, and endometrial tissue. The mechanism of the protective effect of progesterone in ovarian cancer is not known and it is unclear if the RANK/RANKL signaling pathway plays a role in this effect. There are ongoing trials investigating the use of denosumab as chemoprevention for breast cancer in *BRCA1/2* mutation carriers. Additionally, the ABCSG-18 trial studied the use of adjuvant denosumab in the treatment of postmenopausal women with early stage hormone receptor-positive breast cancer receiving aromatase inhibitors. In this phase III trial, an interim analysis reported at the San Antonio Breast Cancer Symposium in 2015 showed that adjuvant denosumab had a trend towards improved disease free survival (hazard ratio [HR]=0.816, p=0.051).[48] This suggests that effects of denosumab in breast cancer may be applied outside of *BRCA1/2* mutation carriers.

Rationale for dosing of denosumab

In this study, we will be administering denosumab 120 mg every 4 weeks for a total of 1-2 doses. This is the dose used in patients with bone metastases from solid tumor malignancies and has a good safety profile.[42,43] Additionally, this is the dose being investigated in an ongoing pre-surgical window of opportunity study in normal breast tissue of *BRCA1/2* mutation carriers. In this window of opportunity study, patients are given 120 mg denosumab monthly for 3 months and the primary endpoint is change in proliferation in breast tumor tissue as measured by Ki-67 staining.[38] Based on this dosing regimen, we have decided to investigate the effect of denosumab 120 mg every 4 weeks for a total of 1-2 doses on the proliferation rate of the fimbrial end of the fallopian tube. While the optimal number of doses to reach steady state levels is not certain, we believe that one dose should allow for sufficient circulating levels of the drug. A second dose of denosumab will be allowed in order to ensure a dose is delivered within 4 weeks of scheduled surgery. Since participants in the study will be undergoing risk-reducing salpingo-oophorectomy during the luteal phase of the menstrual cycle, in order to ensure consistent exposure to denosumab across all participants in the treatment arm, the first dose of denosumab will be administered within the first 3 days of the menstrual cycle.

This proposed study will address whether denosumab alters proliferation in the fimbrial end of the fallopian tube, the presumed site of origin of HGSC in *BRCA1/2* mutation carriers. Given the high risk for ovarian cancer in this population, high mortality associated with the diagnosis, and limited options for ovarian cancer prevention and screening, there is a large unmet need for an ovarian cancer chemopreventive agent. The purpose of this pilot study is to assess the biomarker effects of denosumab in premenopausal women scheduled for risk-reducing salpingo-oophorectomy with or without hysterectomy. Our primary objective is to determine whether there is a difference (either an increase or decrease) in Ki67 proliferation index in the fimbrial end of the fallopian tube among premenopausal *BRCA1/2* mutation carriers after denosumab treatment compared to no treatment. Even if there is no difference in Ki67 expression, demonstrating no detrimental effect of denosumab on proliferation in the ovaries and fallopian tubes would be of interest, as this agent is actively being investigated for breast cancer prevention among female mutation carriers.

Rationale for including Alcohol and Tobacco Assessment Questionnaires

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is

including assessment of tobacco and alcohol use at baseline and (include follow-up timepoint), to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

2.3 Rationale for Biomarkers

Tissue proliferation rate (Ki67) and apoptosis (cleaved caspase-3)
Ki67 expression as a marker of proliferation has been associated with progression to malignancy and thus may be used as a measure of a tissue's risk for becoming dysplastic.[49] Nolan *et al.* demonstrated that the mitogenic response of *BRCA1*$^{mut/+}$ breast tissue to exogenous progesterone, as measured by Ki67, was more pronounced than in wild-type breast tissue. Additionally, when the *BRCA1*$^{mut/+}$ breast tissue was concomitantly exposed to denosumab and progesterone, the progesterone-induced increase in Ki67+ cells was blocked.[38]

In the fallopian tube epithelium (FTE), Ki67 expression has been shown to be increased globally in women with *BRCA1* mutations (when compared to average-risk women)[50,51]; however, another study suggested that Ki67 is increased in the follicular phase when compared to the luteal phase and is not dependent on *BRCA* mutation status.[52] Norquist *et al.* hypothesized that the haploinsufficiency of *BRCA1* leads to increased tubal epithelial proliferation and this proliferation does not decrease appropriately with advancing age, as it does in average-risk women. This increased proliferation allows for the clonal expansion of tubal cells with random *TP53* mutations and subsequently neoplastic potential.[50] George *et al.* demonstrated that there is an increase in Ki67 expression in FTE cells starting from benign cells to disease development.[52] Kuhn *et al.* also demonstrated that serous tubal intraepithelial carcinoma (STIC) and HGSC have higher Ki67 expression than normal FTE.[53] In our proposal, our aim is to assess the impact of RANKL inhibition with denosumab on the proliferation rate of the fimbrial tissue (primary endpoint) and ovarian and endometrial epithelium [if also undergoing hysterectomy] (secondary endpoints). Ki67 expression is readily determined by immunohistochemistry (IHC) of formalin-fixed paraffin-embedded (FFPE) tissue. Since there is variation in Ki67 expression in the FTE depending on phase of menstrual cycle (luteal versus follicular), all participants in the study will undergo risk-reducing salpingo-oophorectomy during the luteal phase of the menstrual cycle for consistency. We will assess whether denosumab exposure affects cell death in the fimbrial tissue, ovarian epithelium, and endometrium (if also undergoing hysterectomy). Cleaved caspase-3 is a marker of apoptosis and can be readily determined with IHC of stained tissue sections.

RANK/RANKL tissue expression
As described above, RANK/RANKL signaling has been shown to play a role in mammary tumorigenesis and RANK/RANKL expression has been demonstrated in breast cancer tumors. There are no published reports of the role of RANK/RANKL signaling in ovarian cancer. This study will assess for the presence of RANK and RANKL protein expression (IHC) in fimbrial tissue, ovarian epithelium, and endometrium (if also undergoing hysterectomy).

ER/PR tissue expression
In normal fimbrial tissue, there is strong ER expression in the early/mid-follicular phase, gradually declining to faint expression at around the time of ovulation. During the luteal phase, there is moderate ER expression.[54] In normal fimbrial tissue, PR staining showed greater variability in the follicular phase and decreased expression in the late luteal phase.[54] In breast tissue, exposure to denosumab significantly reduced colony formation in the ER-/PR- luminal progenitor subset which express RANK.[38] We will assess if ER/PR expression in fimbrial tissue, ovarian epithelium, and endometrium (if also undergoing hysterectomy) differs after denosumab treatment versus no treatment and whether ER/PR expression is associated with Ki67 proliferation index.

CD44 and p53 tissue expression
CD44 is a hyaluronate receptor and a putative marker of epithelial progenitor cells in the fallopian tube and has been shown to be concentrated in the distal fimbria.[55,56] Tiourin *et al.* showed that there is a significant reduction in CD44+ progenitors as well as a reduction in proliferation (Ki67) of progenitors in the distal fallopian tube epithelium with tubal ligation.[49] We will assess if there is a reduction in CD44+ progenitors in the fimbrial tissue, ovarian epithelium, and endometrium (if also undergoing hysterectomy) after denosumab treatment versus no treatment.

Mutation in *TP53* is seen in the vast majority of high grade serous carcinomas and is thought to be an early event in carcinogenesis.[57-59] The p53 signature is the staining of p53 of a consecutive number of nuclei in the fallopian tube epithelium by IHC and is thought to be a precursor of serous tubal intraepithelial carcinoma (STIC).[60] We will assess if denosumab versus no treatment affects p53 expression in fallopian tube epithelium, ovarian epithelium, and endometrium (if also undergoing hysterectomy).

STAT3 and pSTAT3 tissue expression
STAT3 plays a key role in cell growth, apoptosis, transformation, differentiation, migration, and cell survival.[61] It becomes phosphorylated in response to cytokines and growth factors. George *et al*. have demonstrated that STAT3 expression is higher in *BRCA1* mutated fallopian tube epithelium as compared to non-*BRCA1* mutated fallopian tube epithelium.[62] We will assess if there is a reduction in STAT3 and pSTAT3 in the fimbrial tissue, ovarian epithelium, and endometrium (if also undergoing hysterectomy) after denosumab treatment versus no treatment.

Tissue gene expression profiling
In RANK+ luminal progenitor (LP) cells of breast tissue, there is upregulation of genes implicated in proliferation, including *TOP2A, MKI67, PBK, and CDK1*. Expression of these genes is markedly increased in RANK+ LP cells from $BRCA1^{mut}$ breast tissue when compared to wild-type breast tissue.[38] Additionally, gene expression analyses have revealed that there is a significant elevation of inflammation or immune related mRNA in the normal fallopian tube of *BRCA1* mutation carriers.[52] We will look for enrichment of genes involved in the RANKL, cell proliferation, cell cycle progression, and inflammation pathways in fimbrial tissue and ovarian epithelium.

Gene expression profiling will be performed with the nCounter™ PanCancer Pathways Panel by nanoString Technologies on fimbrial and ovarian tissue of participants from both the treatment and control arms. The system can directly assay tissue to analyze 770 essential genes representing 13 canonical pathways, including cell cycle, apoptosis, and DNA damage control pathways. This assay can use FFPE-derived and purified total RNA and does not require reverse transcriptase reactions. Results of this assay using FFPE-derived tissue were highly correlated with purified total RNA from fresh tissue (R^2>0.97). The assay demonstrates a high level of sensitivity and precision even at very low levels of expression.

Serum progesterone and estradiol levels
BRCA1/2 carriers have higher titers of serum estrogen and progesterone during the luteal phase as compared to non-mutation carriers.[23] In breast tissue, progesterone stimulates the secretion of RANKL, which binds to RANK in luminal progenitor cells and stimulates mammary epithelial cells to proliferate.[40,41] We will explore if there is variation in progesterone and estradiol levels based on exposure to denosumab and if Ki67 expression in fimbrial tissue is associated with serum progesterone and estradiol levels.

Serum denosumab level

Serum denosumab level will be checked at baseline and on the day of surgery. We will assess the impact of denosumab drug levels on the other serum and tissue biomarkers.

Serum C-terminal telopeptide (CTX) level

The use of denosumab for osteoporosis and solid tumor bone metastases results in suppressed bone turnover as indicated by decreased serum CTX.[44,63] There is an increase in bone turnover after surgical menopause, including an increase in CTX.[64] The serum CTX level was found to increase above baseline serum CTX level within 3 months after discontinuation of denosumab and peak at 6 months after discontinuation of denosumab in postmenopausal women who received 24 months of denosumab 60 mg every 6 months for osteoporosis (**Figure 3**).[45] We will assess changes in CTX over the course of denosumab treatment (baseline and time of surgery) and in follow-up (6 and 12 months after start of intervention) after undergoing risk-reducing surgery. We would expect the bone turnover marker to be affected by surgical menopause and increase within 6 to 9 months after discontinuation of denosumab treatment.

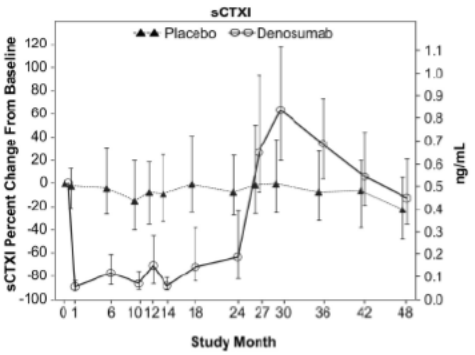

Figure 3. Trajectory of CTX during denosumab treatment versus placebo (months 0-24) and after discontinuation (months 24-48) in postmenopausal women with osteoporosis[45]

3. SUMMARY OF STUDY PLAN

We will conduct a multicenter, open-label randomized controlled trial of presurgical administration of denosumab versus no treatment among premenopausal women with *BRCA1* or *BRCA2* mutations undergoing risk-reducing salpingo-oophorectomy, with or without hysterectomy. A total of 60 women will be randomized 1:1 to two arms: Arm 1) One dose of 120 mg denosumab subcutaneously (Dose #1 within the first 3 days of the menstrual cycle), followed by a possible Dose #2 4-weeks later (in the case that surgery cannot be performed within 4 weeks of Dose #1), for a total of 1-2 doses, or Arm 2) No treatment. Stratification factors include: 1) *BRCA1* versus *BRCA2* mutation status and 2) Use of hormonal contraceptives within the past 3 months (yes/no). Since hormonal contraceptive use will affect serum progesterone level, we will stratify based on the use of hormonal contraceptives within the past 3 months prior to registration. We will also collect information on prior hormonal contraceptive use, duration of use, and prior bilateral tubal ligation (which are known to influence ovarian cancer risk) on the case report forms. Assuming a 10% unevaluable rate, we expect to have 54 evaluable participants (27 per arm). An interim analysis will be performed after the first 10 participants are enrolled on the study.

Women will undergo baseline clinical assessment, urine or serum pregnancy test, and blood collection for clinical labs and circulating biomarkers. Women randomized to active treatment will receive Dose #1 of 120 mg denosumab within the first 3 days of their menstrual cycle. A subsequent dose (Dose #2) may be given in 4 weeks in the case that surgery cannot be performed within 4 weeks from Dose #1. All participants will be given a 6-month supply of oral calcium 1000 mg (two 500 mg tablets) daily and vitamin D3 1000 IU daily to reduce the risk of hypocalcemia to be started within the first 3 days of their menstrual cycle. Study personnel will call participants 1 week after start of drug administration (calcium and vitamin D with or without denosumab) for toxicity assessment and calcium/vitamin D supplement pill compliance. Participants will return for surgery during the luteal phase of the same menstrual cycle (Days 14-28) of the last dose of denosumab (Dose #1 or 2). Participants not receiving denosumab treatment will return for surgery during the luteal phase of a menstrual cycle (Days 14-28) within 2-8 weeks after initiating vitamin D and calcium supplement. For women with irregular periods or menstrual cycles of >28 days, the luteal

phase will be estimated as the second half of the menstrual cycle. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), first dose of denosumab and surgery do not need to be timed with menses as these patients will be in the luteal phase at all times. On the day of surgery for risk-reducing salpingo-oophorectomy with or without hysterectomy, physical exam/vitals (within 5 days prior to surgery), concomitant medications, toxicity assessment, calcium/vitamin D supplement pill compliance, blood collection for clinical labs (within 3 days prior to surgery) and research circulating biomarkers, and tissue collection will be performed.

Additional assessments will occur at months 1-5 (+/- 1 week) for calcium/vitamin D supplement pill compliance, 7-14 days post-surgery for calcium/vitamin D supplement pill compliance by telephone contact, month 6 (+/- 1 week) for toxicity assessment and calcium/vitamin D supplement pill compliance by telephone contact, and clinic visits at months 6 and 12 (+/- 90 days) for toxicity assessment and research blood draw for serum CTX level. Collection of calcium/vitamin D pill bottles will occur at month 6 visit.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Participants must be female, ≥18 years of age, and premenopausal (defined as < 3 months since last menstrual period OR serum follicle-stimulating hormone (FSH) < 20 mIU/mL).

4.1.2 Documented germline pathogenic or likely pathogenic variant in the *BRCA1* or *BRCA2* genes.

4.1.3 Participants must be scheduled for or in the process of scheduling a risk-reducing salpingo-oophorectomy with or without hysterectomy – either bilateral or unilateral (if prior unilateral oophorectomy or salpingectomy for benign condition).

4.1.4 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A).

4.1.5 Participants must have normal organ and marrow function as defined below:

Leukocytes	≥ 3,000/microliter
Absolute neutrophil count	≥ 1,500/microliter
Platelets	≥ 100,000/microliter
Total bilirubin	≤ 2 x institutional upper limit of normal (ULN)
AST (SGOT)/ALT (SGPT)	≤ 1.5 x institutional ULN
Creatinine clearance	≥ 30 mL/min
Serum calcium or albumin adjusted	≥ 8.0 mg/dL and ≤ 11.5 mg/dL

4.1.6 Participant must have a negative urine or serum pregnancy test 14 days prior to randomization or drug administration. The effects of denosumab on the developing human fetus at the recommended therapeutic dose may cause fetal harm when administered to pregnant women. Women of child-bearing potential must agree to use adequate contraception from time of drug administration to time of surgery. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.7 Women currently on hormonal contraception (*i.e.,* oral contraceptives, Mirena® intrauterine device [IUD]) are eligible to participate if they have been on a stable dose for at least 3 months. Women who have undergone bilateral tubal ligation are also eligible to participate in this study. There will be stratification for hormonal contraceptive use within 3 months prior to registration.

4.1.8 Participants must be willing to take supplemental oral calcium 1000 mg (two 500 mg tablets) and vitamin D3 1000 IU daily for six months (which will be supplied by the research study) after receiving denosumab treatment or no treatment.

4.1.9 Ability to understand and the willingness to sign a written informed consent document in English or Spanish or Hebrew.

4.1.10 Participants must have a dental examination ≤ 6 months of study registration.

4.1.11 Willing to not undergo any other elective surgery procedure with general anesthesia or conscious sedation during the treatment period. The treatment period is completed after the last injection of denosumab is administered.

4.2 Exclusion Criteria

4.2.1 History of ovarian cancer. History of breast cancer or any other malignancy is permitted if last chemotherapy treatment was greater than 6 months prior to registration and participant is not using endocrine therapy (as per 4.2.7).

4.2.2 Previous treatment with denosumab (including Prolia for osteoporosis or Xgeva for bone metastases) or use of bisphosphonate within 3 months of registration to the study.

4.2.3 Participants receiving any other investigational agents.

4.2.4 History of allergic reactions or hypersensitivity attributed to denosumab or any components of denosumab or compounds of similar chemical or biologic composition to denosumab, such as other RANKL inhibitors.

4.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.6 Pregnant and breastfeeding women are excluded from this study because denosumab is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events (AEs) in nursing infants secondary to treatment of the mother with denosumab, breastfeeding should be discontinued if the mother is treated with denosumab. There is no minimum amount of time since pregnancy/breastfeeding required before enrolling into the study. However, the date of delivery, pregnancy termination, or weaning from breast-feeding will be documented on case report forms. Female subjects of child bearing potential and not willing to use, in combination with her partner, highly effective contraception during treatment will be excluded.

4.2.7 Use of endocrine therapy (selective estrogen receptor modulator, aromatase inhibitor, GnRH agonist) within 6 months of registration to the study.

4.2.8 Prior history or current evidence of osteonecrosis or osteomyelitis of the jaw, evidence of untreated local gum or oral infection, or non-healed dental or oral surgery.

4.2.9 Active dental or jaw conditions which require oral surgery/dental procedures, including tooth extraction within 6 months of the registration to the study. Dental fillings are permitted within 6 months of study registration.

4.2.10 Other risk factors for the development of osteonecrosis of the jaw (ONJ) including poor oral hygiene, use of a dental appliance, immunosuppressive therapy, treatment with angiogenesis inhibitors, systemic corticosteroids, diabetes, or gingival infections.

4.2.11 Known sensitivity to any of the products to be administered during the study (*e.g.,* calcium or vitamin D).

4.2.12 Known serious infections, including a history of active hepatitis B, hepatitis C, or HIV. Screening for these infections is not required for study enrollment.

4.2.13 Hypocalcemia (serum calcium or albumin adjusted calcium < 8.0 mg/dL) or renal dysfunction (creatinine clearance < 30 mL/min).

4.2.14 Women with known osteoporosis or history of osteoporotic (fragility) fracture of the spine.

4.3 Inclusion of Women and Minorities

Participants will be adult women of all races and ethnic groups who are at least 18 years old. Male participants will not be recruited as the primary objective is to determine the effect of denosumab on fallopian tube fimbrial cells. Children will not be recruited into the trial because risk-reducing surgery is not applicable in the pediatric population.

Our goal is to ensure that the study is available to women of all races and ethnic groups. At US sites, efforts will be made to enroll women from diverse racial, ethnic and socio-economic backgrounds. Of note, *BRCA1/2* mutations have a prevalence of 1:400 among most racial/ethnic groups and a prevalence of 1:40 among those of Ashkenazi Jewish descent.[1,2]

4.4 Recruitment and Retention Plan

This multicenter protocol will be conducted at the following five sites: Columbia University Irving Medical Center (CUIMC), New York, NY; Weill-Cornell Medical Center, New York, NY; the Dana Farber Cancer Institute (DFCI), Boston, MA; and Tel Aviv Sourasky Medical Center and Chaim Sheba Medical Center, Tel Aviv, Israel. CUIMC will be the Lead Site. The rates of risk-reducing salpingo-oophorectomies at these sites will allow the recruitment of the required sample size. Efforts will be made to enroll women from diverse ethnic and socio-economic backgrounds. Prior to enrollment on the study, the physician will discuss the study protocol in detail with the participant, including possible toxicities. The informed consent document will be reviewed by study personnel with the participant. All participants will be offered compensation for parking, time and travel for each of the following study-related visits ($30 or the amount needed to cover parking expenses at the time of each study-related visit, depending on the site): baseline visit, Dose #1 denosumab (treatment group) or dispensing of vitamin D/calcium supplement (no treatment group), and Dose #2 denosumab (treatment group, if administrered). To facilitate accrual, information about the trial will be advertised, for example, on the websites of participating institutions. Recruitment and retention efforts will be evaluated routinely by the site coordinator and the study staff. The study recruitment and retention plan will be modified as necessary to promote rapid accrual. Please refer to the study-specific recruitment and retention plan for more details. The domestic and international planned enrollment tables are shown below.

Racial Categories	DOMESTIC PLANNED ENROLLMENT REPORT				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	2	0	0	0	2
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	5	0	2	0	7
White	31	0	6	0	37
More Than One Race	2	0	0	0	2
Total	40	0	8	0	48

Racial Categories	INTERNATIONAL (including Canadian participants) PLANNED ENROLLMENT REPORT				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	9	0	0	0	9
More Than One Race	3	0	0	0	3
Total	12	0	0	0	12

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

Denosumab 120 mg every 4 weeks will be administered subcutaneously in a series of 1-2 doses prior to surgery. The first dose will be administered within the first 3 days of the menstrual cycle. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), first dose of denosumab does not need to be timed with menses. The subsequent dose (possible) will be given in 4 weeks if the surgery cannot be performed within 4 weeks of Dose #1 of denosumab. Participants will receive 1-2 doses to ensure that a dose of denosumab is administered within 4 weeks of surgery. There will be a no treatment control arm.

All participants will be given a 6-month supply of oral calcium 1000 mg (two 500 mg tablets) daily and oral vitamin D3 1000 IU daily, which will be started within the first 3 days of the same menstrual cycle with denosumab dose #1 (treatment arm) or no treatment. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), first dose of denosumab and initiation of calcium/vitamin D supplement pills do not need to be timed with menses. Calcium will be dosed at 1000 mg (two 500 mg tablets) and Vitamin D3 will be dosed at 1000 IU (1 tablet). The calcium and Vitamin D3 supplements should each be taken once daily within a 12-hour window. If a dose is not taken within the 12-

hour window, it will be considered a missed dose and should be resumed the next day. There is no minimum amount of time required between doses. If a dose is vomited, it does not need to be repeated. Tablets may be crushed, chewed, or dissolved in water. Tablets may be taken with or without food. There are no foods that need to be avoided while taking the calcium and vitamin D3 supplements. These instructions will be given in writing to the participant ("Calcium and Vitamin D3 Tablet Information" sheet found in Appendix E).

5.2 Denosumab Administration

According to the package insert for denosumab, it should be visually inspected for particulate matter and discoloration prior to administration. Denosumab is a clear, colorless to pale yellow solution that may contain trace amounts of translucent to white proteinaceous particles. Denosumab should not be used if the solution is discolored or cloudy or if the solution contains many particles or foreign particulate matter. Prior to administration, denosumab may be removed from the refrigerator and brought to room temperature (up to 25°C/77°F) by standing in the original container. This generally takes 15 to 30 minutes. Denosumab should not be warmed in any other way. A 27-gauge needle may be used to withdraw and inject the entire contents of the vial and the vial should be discarded after single-dose or entry. A healthcare provider approved at each institution to deliver the injection will administer the injection in a sterile fashion in the clinical facility at each study site. The 1.7 mL by volume (120 mg) injection of denosumab will be administered subcutaneously in the upper arm, upper thigh, or abdomen. The healthcare provider will determine the best site for drug administration and will rotate the injection site on subsequent visits. Each site will be responsible for furnishing the 25-to-27-gauge ½-inch to 5/8-inch in length needle to administer the drug injections. The injection will be administered within the first 3 days of the participant's menstrual cycle (Dose #1) with a possible dose (Dose #2) 4 weeks after that for a total of 1-2 doses. Dose #2 will be administered if the surgery cannot be performed within 4 weeks of Dose #1 of denosumab. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), first dose of denosumab does not need to be timed with menses. The participant will be monitored by clinical observation for 30 minutes after each injection to assess for any acute or anaphylactic reactions, such as rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat.

5.3 Run-in Procedures

This trial will not include a run-in period.

5.4 Contraindications

Eligible participants should not have specific contraindication to denosumab. Any pre-existing dental condition should be carefully evaluated before enrolling the participants. Evidence of hypocalcemia or renal dysfunction on baseline laboratory evaluation will exclude participation in the study.

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included. Women already taking calcium or vitamin D supplementation will be asked to stop taking these supplements during the 6-month study period, because these supplements will be provided for all study participants.

5.6 Dose Modification

Participants will be asked to receive full dose treatment. Due to the short duration of treatment with denosumab, no dose modification will be applied. Criteria for withholding a dose of denosumab due to adverse effects and when to resume denosumab is described in Section 7.3.

5.7 Adherence/Compliance

Compliance is defined as receiving 1-2 doses of 120 mg denosumab and undergoing risk-reducing salpingo-oophorectomy (bilateral or unilateral). Assuming a 10% unevaluable rate (*e.g.,* insufficient fimbrial tissue for Ki67 assessment), our goal is to randomize a total of 60 participants to yield at least 54 evaluable participants. Those who do not undergo risk-reducing salpingo-oophorectomy will be replaced, so additional participants may be randomized.

For the primary endpoint of proliferation rate (Ki67) in the fimbrial end of the fallopian tube, "evaluable" will be defined as receiving at least 1 dose of denosumab (in the intervention arm) or no treatment (in the control arm) and undergoing risk-reducing salpingo-oophorectomy (in both arms). For the safety endpoint, all participants who receive denosumab treatment or no treatment will be included in the safety evaluation. For all secondary endpoints, all non-compliant participants will be analyzed according to the intent-to-treat principle. All levels of compliance will be included in the full evaluation of endpoints if post-treatment tissue and paired serum samples are available.

Compliance to calcium/vitamin D pill supplements will be conducted at 1 week after start of supplements (by telephone contact), at 1, 2, 3, 4, 5, and 6 months after start of supplements (by telephone contact or in person), at time of surgery (in person), and at 1 week post-surgery (by telephone contact). The procedures for assessing compliance are in Appendix E (Assessment of calcium and vitamin D compliance). At the 6 month visit, pill bottles and remaining pills will be collected from participant.

6. PHARMACEUTICAL INFORMATION

6.1 Denosumab (*IND #[redacted], NCI/DCP*)

Denosumab, a human IgG2 monoclonal antibody directed against the receptor activator of nuclear factor kappa beta ligand (RANKL) is marketed under the trade names Xgeva® or Prolia®, which differ in dosage and strength. This study will use Xgeva®, a sterile, preservative-free, clear, and colorless to pale yellow solution containing 120 mg denosumab/1.7mL in a single-use vial for subcutaneous injection. The drug has an approximate molecular weight of 147 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells. It is approved at a dose of 120 mg every 4 weeks for the prevention of skeletal-related events in patients with bone metastases from solid tumors. It is also indicated for the treatment of giant cell tumor of bone (unresectable or where surgical resection is likely to result in severe morbidity) and hypercalcemia of malignancy refractory to bisphosphonate therapy. For the latter two conditions, Xgeva® is administered every 4 weeks with additional doses (120 mg) on Days 8 and 15 of the first month of therapy. The study agent should not be exposed to direct light or temperatures above 77°F.

6.2 Reported Adverse Events and Potential Risks

The most common adverse reactions in patients (per-patient incidence ≥25%) who received Xgeva® were fatigue/asthenia, hypophosphatemia, and nausea.[65] The most common serious adverse reaction was

dyspnea. The most common adverse reactions resulting in discontinuation of Xgeva® were osteonecrosis and hypocalcemia. Warnings and Precautions in the package insert include severe symptomatic hypocalcemia (fatal cases have been reported), osteonecrosis of the jaw (ONJ), hypersensitivity, atypical subtrochanteric and diaphyseal femoral fracture, and clinically significant hypercalcemia following treatment discontinuation in patients with growing skeletons. The risk of atypical femoral fracture continues after stopping therapy and increases with longer duration of treatment with denosumab. Some patients had fractures up to 9 months after treatment when denosumab was discontinued.

Based on data from nonclinical studies and the mechanism of action, Xgeva® can cause fetal harm when administered to a pregnant women. In animal reproduction studies, administration of denosumab to cynomolgus monkeys throughout pregnancy at a dose 25-fold higher than the recommended human dose of Xgeva® based on body weight resulted in increased fetal loss, stillbirths, and postnatal mortality, along with evidence of absent peripheral lymph nodes, abnormal bone growth, and decreased neonatal growth. Exposure to Xgeva® during pregnancy or within five mots prior to conception can result in fetal harm.

Summarized below are safety data from clinical trials of Xgeva® in approved indications and settings.

Bone Metastasis from Solid Tumors: The safety of Xgeva® for the prevention of skeletal-related events in patients with bone metastasis from solid tumors or lytic bony lesions from multiple myeloma was evaluated in three international randomized, double-blind, active-controlled, noninferiority trials recruiting over 5000 patients. In all three trials, patients were randomized to receive 120 mg Xgeva® subcutaneously every 4 weeks or 4 mg zoledronic acid intravenously (iv) every 4 weeks. The most common adverse reactions (per-patient incidence ≥25%) in patients who received Xgeva® were fatigue/asthenia, hypophosphatemia, and nausea. Severe hypocalcemia (corrected serum calcium <7 mg/dL or <1.75 mmol/L) were also noted at an increased incidence in the denosumab group (3.1%) compared with the zoledronic group (1.3%). Severe hypophosphatemia also occurred in 15.4% of patients treated with Xgeva® compared with 7.4% of patients treated with zoledronic acid. ONJ occurred in 1.8% of patients in the Xgeva® group compared with 1.3% of patients in the zoledronic acid group. Atypical femoral fracture has been reported with Xgeva®.

Giant Cell Tumor of Bone: Single- arm trials evaluated the safety of Xgeva® in about 300 adults or skeletally mature adolescent patients with giant cell tumor of bone who received at least one dose of Xgeva®. The most common adverse reactions in patients (per-patient incidence ≥10%) were arthralgia, headache, nausea, back pain, fatigue, and pain in extremity. The most common serious adverse reactions were osteonecrosis of the jaw (ONJ) and osteomyelitis (per-patient incidence of 0.7%). The most common adverse reactions resulting in discontinuation of Xgeva® were ONJ (per-patient incidence of 0.7%), and tooth abscess or tooth infection (per-patient incidence of 0.7%). Moderate hypocalcemia and severe hypophosphatemia occurred in 2.6% and 9.5% of patients, respectively. The adverse reaction profile appeared similar in skeletally mature adolescents (13–17 years of age).

Hypercalcemia of Malignancy: The adverse reaction profile of Xgeva® was also evaluated in an open-label, single-arm trial in which 33 patients with hypercalcemia of malignancy (with or without bone metastases) refractory to treatment with iv bisphosphonate therapy were enrolled. Adverse reactions occurring in >20% of patients were nausea (30%), dyspnea (27%), decreased appetite (24%),

headache (24%), peripheral edema (24%), vomiting (24%), anemia (21%), constipation (21%), and diarrhea (21%). The following adverse reactions of grade 3 or greater severity considered related to therapy were reported on study: fatigue (3%) and infection (6%). Grade 3 laboratory abnormalities included hypomagnesemia (3%), hypokalemia (3%), and hypophosphatemia (76%). No deaths on study were related to Xgeva® therapy.

In addition to the previously described approved indications, Xgeva® is also being investigated in other settings, as detailed in the Investigator's Brochure [Investigator's Brochure Denosumab (AMG 162) Cancer-related Bone Disease] and discussed briefly below:

Prevention of Bone Metastasis in Subjects with Cancer: The clinical program evaluating Xgeva® in the prevention of bone metastasis in subjects with advanced malignancies includes two studies. The first study (20050147) was a phase 3, randomized, double-blind, placebo-controlled study in men with hormone refractory castration-resistant prostate cancer (CRPC). Adverse drug reactions such as hypocalcemia and ONJ (4.6%) were consistent with the drug's mechanism of action, as reported in the package insert, and no new risks were identified. Incidences of adverse events, serious adverse events, fatal adverse events, and grade 3 to 5 adverse events were similar between the Xgeva® and placebo groups and reflective of underlying disease. Fatal adverse events were generally associated with disease progression or age-related comorbidities. The second study (20060359) is a phase 3, randomized, double-blind, placebo-controlled study in women with early stage breast cancer evaluating Xgeva® as a potential treatment for the prevention of bone metastasis and extraosseous disease recurrence. Results are currently unavailable as the study is ongoing.

Non-Small Cell Lung Cancer (NSCLC): Study 20120249 was a randomized phase 2, double blind, placebo-controlled study of Xgeva® in subjects with untreated Stage IV NSCLC with or without bone metastasis (n=226). Safety results were comparable to those seen in other Xgeva® studies. There were four events of ONJ and one of hypocalcemia in the Xgeva® group.

Multiple Myeloma: Clinical studies have been conducted to evaluate Xgeva® prevention of skeletal-related events (SREs) in multiple myeloma. Study 20090482 comparing Xgeva® to zolendronic acid was designed to be an adequately powered phase 3 study with appropriate stratification of key multiple baseline prognostic factors for survival as well as SREs and anti-myeloma treatments together with myeloma disease monitoring. Overall, adverse event incidences were similar between the two treatment groups. Safety results from this study were generally comparable to those seen in previous SRE studies. Subject incidence of adverse events of hypocalcemia was 16.9% in the Xgeva® group and 12.4% in the zoledronic acid group. There were 35 (4.1%) and 24 (2.8%) positively adjudicated events of ONJ in the Xgeva® and zoledronic acid groups, respectively.

Post-marketing Experience: Adverse reactions identified from post-marketing experience include severe hypocalcemia, musculoskeletal pain, and hypersensitivity. Please refer to the Investigator's Brochure for details.

6.3 Availability

Denosumab is an investigational agent supplied to investigators by NCI, DCP. The calcium 1000 mg (two 500 mg tablets) and vitamin D 1000 IU supplements will also be supplied by NCI, DCP.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of Institutional Review Board approval of the DCP-approved protocol and consent is provided to DCP, and the collection of all essential documents is complete (see DCP website for description of essential documents).

NCI, DCP-supplied agents may be requested by the investigator (or their authorized designees) at each organization. DCP guidelines require that the agent be shipped directly to the institution or site where it will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (including complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. This responsibility has been delegated to the site pharmacists. Include on receipt record: from whom the agent was received, to whom study agent was shipped, date, quantity, and batch or lot number. On the dispensing record, note the quantities and the dates the study agent was administered to each participant.

6.6 Packaging and Labeling

Denosumab and the oral calcium/vitamin D supplements will be packaged and labeled by NCI, DCP.

6.7 Storage

Denosumab is supplied in single-dose vials and a 6-month supply of oral calcium/vitamin D supplements will be dispensed, and should be stored in a secure location at temperatures between 2°C to 8°C (36°F to 46°F).

6.8 Registration/Randomization

Screening and Registration into the DMI Database:
Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's ID (PID) upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant. Refer to **Section 13.2** for details of randomization.

Screening/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff needs to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF; 2) the Randomization CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious Adverse Event during the screening process, a Serious Adverse Event (SAE) form must be completed.

6.9 Blinding and Unblinding Methods

Not applicable

6.10 Agent Destruction/Disposal

DCP-supplied agents: at the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List".

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1.1 Schedule of Events: Denosumab Arm

	Baseline/ Registration (within 30 days prior to randomization)	Randomization	Dose #1 of denosumab given on Day 1-3 of menstrual cycle[n]	Day 8-10 of menstrual cycle	Doses #2 of denosumab (possible)	Surgery[a,n]	7-14 days post-surgery	Month 1-5 from Dose #1 calls	6 months from Dose #1 visit	12 months from Dose #1 visit
Window (days)					+/- 3 days			+/- 7 days	+/- 90 days	+/- 90 days
Informed consent	X									
Physical exam	X[b]					X[c]				
Oral examination[d]	X		X		X	X[m]				
Dental examination[d]	X									
All concurrent medications	X					X[q]				
Confirm eligibility	X									
Randomization		X[e]								
Toxicity assessment				X[f]	X	X[q]			X	X
Clinical blood draw[g]	X		X[h]		X[h]	X[r]				
Urine or serum pregnancy test	X[p]		X[p]		X[p]					
Serum FSH	X									
Denosumab administration[i]			X		X					
Dispensing of calcium/vitamin D			X							
Collect unused study agent									X	
Calcium/vitamin D pill compliance assessment				X[f]	X[o]	X[o,q]	X[f]	X[f,l]	X[o]	
Research blood collection	X					X			X	X
Serum progesterone	X					X				
Serum estradiol	X					X				
Serum denosumab	X					X				
Serum CTX	X					X			X	X
Research tissue collection (fimbria, ovarian epithelium, endometrium [if undergoing hysterectomy])						X				
Ki67 (IHC)						X				
RANK/RANKL						X				

(IHC)										
Cleaved caspase-3 (IHC)						X				
ER/PR (IHC)						X				
CD44/p53 (IHC)						X				
STAT3/pSTAT3 (IHC)						X				
Gene expression profiling (only on fimbrial and ovarian tissue)						X				
Tobacco and Alcohol Use Assessment	X[j]									X[k]

Abbreviations: CBC=complete blood count; ER=estrogen receptor; IHC=immunohistochemistry; LFTs=liver function tests; PR=progesterone receptor; RANK=receptor activator of NF-KB; RANKL=receptor activator of NF-KB ligand

a. Risk-reducing salpingo-oophorectomy (bilateral or unilateral) will be scheduled during the luteal phase (day 14-28) of the same menstrual cycle after administration of the last dose of denosumab (Dose #1 or 2). For women with irregular periods or menstrual cycles of >28 days, then the luteal phase will be estimated as the second half of the menstrual cycle. For women who do not cycle, please see Sections 7.3 and 7.4.
b. Full physical examination including vital signs, height, and weight
c. Focused physical examination based on symptomatology and including vital signs and weight. Physical exam may be performed up to 5 days prior to surgery (-5 days).
d. A visual examination of the oral cavity, including teeth, mucosa, and jaws, will be conducted by the investigator, or designated licensed healthcare professional, at screening, to establish baseline oral health conditions, and at follow-up, to identify and document any new abnormalities or changes in pre-existing conditions. If any new abnormalities or changes in pre-existing conditions are identified, the investigator may refer the participant to follow up with a dentist or other oral health specialist. A dental exam is required $\leq$ 6 months of study registration.
e. Randomization can be completed on the same day/visit as baseline evaluation
f. Assessed via telephone contact. Contact may occur over email if patient prefers.
g. HEMATOLOGY: CBC (hemoglobin, hematocrit, RBC, WBC, platelet count) with differential (neutrophils, bands [may be included within neutrophil count or reported separately], lymphocytes, monocytes, eosinophils, basophils)
CHEMISTRY: Na^+, K^+, Cl^-, CO_2, BUN, creatinine, glucose, Ca^{+2}, PO_4, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT); FSH (only at baseline to assess menopausal status)
h. Only Na^+, K^+, Cl^-, CO_2, BUN, creatinine, glucose, Ca^{+2}, PO_4, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, AST (SGOT), and ALT (SGPT) are required with each denosumab injection. Labs prior to first injection only need to be repeated if the first dose of denosumab falls outside of 30-day window from registration.
i. Denosumab is administered every 4 weeks. Dose #2 is given if the surgery cannot be performed within the specified time frame of Dose #1 of denosumab
j. See Appendix B "Alcohol and Tobacco Use Assessment Questionnaires – Baseline"
k. See Appendix C "Alcohol and Tobacco Use Assessment Questionnaires – Month 12". Assessed in person or on the phone.
l. At Month 5 remind the participant to bring pill bottles and remaining pills to Month 6 visit.
m. The oral examination planned at the time of surgery can be performed up to 4 weeks after the surgery.
n. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), the first dose of denosumab and surgery do not need to be timed with menses.

o. In-person contact for calcium/Vitamin D compliance assessment. Compliance assessments for treatment arm participants at Dose 2 of denosumab will replace the telephone compliance assessments at Month 2.
p. Urine or serum pregnancy test must be within 14 days prior to randomization and 14 days prior to each denosumab injection.
q. Telephone contact the day before surgery may be used instead of in person assessment of concurrent medications, toxicity assessment, and calcium/Vitamin D compliance assessment.
r. Only calcium and phosphate levels should be drawn within 3 days of the day of surgery (-3 days). These may also be drawn at the same time as day of surgery research blood draw.

7.1.2 Schedule of Events: Control Arm

	Baseline/ Registration (within 30 days prior to randomization)	**Randomization**	**Day 1-3 of menstrual cycle[a]**	**Day 8-10 of menstrual cycle**	**Surgery [a,b]**	**7-14 days post-surgery**	**Months 1-5 from start of Calcium/ Vitamin D calls**	**6 months from start of Calcium/ Vitamin D visit**	**12 months from start of Calcium/ Vitamin D visit**
Window (days)							+/- 7 days	+/- 90 days	+/- 90 days
Informed consent	X								
Physical exam	X[c]				X[d]				
Oral examination[e]	X				X[f]				
Dental examination[e]	X								
All concurrent medications	X				X[o]				
Confirm eligibility	X								
Randomization		X[g]							
Toxicity assessment				X[h]	X[o]			X	X
Clinical blood draw[i]	X				X[p]				
Urine or serum pregnancy test	X[j]								
Serum FSH	X								
Dispensing of calcium/vitamin D			X						
Collect unused study agent								X	
Calcium/vitamin D pill compliance assessment				X[h]	X[k,o]	X[h]	X[h,l]	X[k]	
Research blood collection	X				X			X	X
Serum progesterone	X				X				
Serum estradiol	X				X				
Serum denosumab	X				X				
Serum CTX	X				X			X	X
Research tissue collection (fimbria, ovarian epithelium, endometrium					X				

[if undergoing hysterectomy])									
Ki67 (IHC)					X				
RANK/RANKL (IHC)					X				
Cleaved caspase-3 (IHC)					X				
ER/PR (IHC)					X				
CD44/p53 (IHC)					X				
STAT3/pSTAT3 (IHC)					X				
Gene expression profiling (only on fimbrial and ovarian tissue)					X				
Tobacco and Alcohol Use Assessment	X[m]								X[n]

Abbreviations: CBC=complete blood count; ER=estrogen receptor; IHC=immunohistochemistry; LFTs=liver function tests; PR=progesterone receptor; RANK=receptor activator of NF-KB; RANKL=receptor activator of NF-KB ligand

a. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), the first dose of calcium/vitamin D and surgery do not need to be timed with menses.
b. Risk-reducing salpingo-oophorectomy (bilateral or unilateral) will be scheduled during the luteal phase (day 14-28) of the menstrual cycle 2-8 weeks after initiation of calcium and vitamin D supplement. For women with irregular periods or menstrual cycles of >28 days, then the luteal phase will be estimated as the second half of the menstrual cycle. For women who do not cycle, please see Sections 7.3 and 7.4.
c. Full physical examination including vital signs, height, and weight
d. Focused physical examination based on symptomatology and including vital signs and weight. Physical exam may be performed up to 5 days prior to surgery (-5 days).
e. A visual examination of the oral cavity, including teeth, mucosa, and jaws, will be conducted by the investigator, or designated licensed healthcare professional, at screening, to establish baseline oral health conditions, and at follow-up, to identify and document any new abnormalities or changes in pre-existing conditions. If any new abnormalities or changes in pre-existing conditions are identified, the investigator may refer the participant to follow up with a dentist or other oral health specialist. A dental exam is required $\leq$ 6 months of study registration.
f. The oral examination planned at the time of surgery can be performed up to 4 weeks after the surgery.
g. Randomization can be completed on the same day/visit as baseline evaluation
h. Assessed via telephone contact. Contact may occur over email if patient prefers.
i. HEMATOLOGY: CBC (hemoglobin, hematocrit, RBC, WBC, platelet count) with differential (neutrophils, bands [may be included within neutrophil count or reported separately], lymphocytes, monocytes, eosinophils, basophils)
   CHEMISTRY: Na^+, K^+, Cl^-, CO_2, BUN, creatinine, glucose, Ca^{+2}, PO_4, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT); FSH (only at baseline to assess menopausal status)
j. Urine or serum pregnancy test must be within 14 days prior to randomization
k. In-person contact for calcium/Vitamin D compliance assessment.

l. At Month 5 remind the participant to bring pill bottles and remaining pills to Month 6 visit.
m. See Appendix B “Alcohol and Tobacco Use Assessment Questionnaires – Baseline”
n. See Appendix C “Alcohol and Tobacco Use Assessment Questionnaires – Month 12”. Assessed in person or on the phone.
o. Telephone contact the day before surgery may be used instead of in person assessment of concurrent medications, toxicity assessment, and calcium/Vitamin D compliance assessment.
p. Only calcium and phosphate levels should be drawn within 3 days of the day of surgery (-3 days). These may also be drawn at the same time as day of surgery research blood draw.

7.2 Baseline Testing/Prestudy Evaluation

Baseline testing/prestudy evaluation will be conducted within 30 days of randomization, may require multiple visits and will consist of the following procedures:

- Informed consent must be obtained prior to starting any further study procedures.
- Registration: Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's ID upon completion of the registration process. Participants will also be registered into site-specific registry databases as applicable.
- Baseline medical history, to include a review of previous medical and surgical history, reproductive history, family history, demographic information, including age and race.
- Pathogenic germline mutation or likely pathogenic variant in the *BRCA1* or *BRCA2* genes must be confirmed by review of genetic test report.
- Use of concomitant medications will be reviewed.
- A physical examination will be done that will include vital signs (temperature, blood pressure, heart rate, respiratory rate).
- An oral examination will be done that will include a visual examination of the oral cavity, including teeth, mucosa, and jaws.
- A dental examination is required $\leq$ 6 months of study registration.
- Height, weight.
- Pre-study blood draw will include the following:
    - Clinical laboratory tests: CBC (hemoglobin, hematocrit, RBC, WBC, platelet count) with differential (neutrophils, bands [may be included within neutrophil count or reported separately], lymphocytes, monocytes, eosinophils, basophils); Chemistry (Na^+, K^+, Cl^-, CO_2, BUN, creatinine, glucose, Ca^{+2}, PO_4, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, AST [SGOT], ALT [SGPT]; FSH [to assess menopausal status])
    - Research blood collection
        - Serum progesterone
        - Serum estradiol
        - Serum denosumab level
        - Serum CTX level
- A urine or serum pregnancy test must be done within 14 days prior to randomization. Results must be known prior to randomization and drug dispensing.
    - If the pregnancy test result is positive at Baseline (any time before randomization), the participant is a Screen Failure.
- Tobacco and Alcohol Use Assessment, using the Baseline questionnaires. See Appendix B "Alcohol and Tobacco Use Assessment Questionnaires – Baseline". Refer to Appendix D for resources for alcohol and tobacco quitting. These resources can be given to individuals if there is concern about alcohol or tobacco dependence. It is not expected that investigators will refer individuals for assistance or that they will undertake the care of individuals for alcohol or tobacco dependence.
- Confirm eligibility: At the completion of the screening period, eligibility must be confirmed. Only after eligibility is confirmed, can eligible participants be randomized.

7.3 Evaluation During Study Intervention

The first denosumab injection (Dose #1) will be administered within the first 3 days of the first or second menstrual cycle after baseline evaluation (treatment arm only). For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), the first dose of denosumab does not need to be timed with menses. In those patients receiving denosumab (treatment arm only), an oral examination will be done prior to Dose #1 that will include a visual examination of the oral cavity, including teeth, mucosa, and jaws. Participant must have a negative urine or serum pregnancy test within 14 days prior to or on the day of Dose #1.

In addition, all participants (control and treatment groups) will be given a 6-month supply of oral calcium and vitamin D supplements. If randomized to treatment group, the first dose of denosumab and calcium and vitamin D supplements will be administered within the first 3 days of the menstrual cycle. If randomized to no treatment group, the calcium and vitamin D supplements will be administered within the estimated first 3 days of the menstrual cycle. For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), the first dose of oral calcium and vitamin D supplements and first dose of denosumab (if applicable) do not need to be timed with menses.

If a participant does not cycle and has FSH < 20 mIU/mL, the date of last menstrual period (LMP) will be documented and the follicular and luteal phases of the participant's menstrual cycle will be estimated based on a 28-day cycle (follicular phase as Days 1-13 and luteal phase as Days 14-28).

One week later (Days 8-10 of menstrual cycle), study personnel will make telephone contact with all participants (control and treatment groups) for toxicity assessment and calcium and vitamin D pill compliance. They will be specifically asked about denosumab-related toxicities, including skin rashes, infections, perioral numbness, tingling in fingers, or groin or thigh pain indicative of atypical fracture. Calcium and vitamin D pill compliance will be assessed every month (+/- 1 week) from start of supplements.

In the treatment arm only, participants may receive a second dose of denosumab (Dose #2) after four weeks (+/- 3 days) from Dose #1 if the surgery cannot be performed within 4 weeks of Dose #1 of denosumab. At each visit for denosumab administration, the following assessments will be performed:

- Study personnel will perform toxicity assessment and assess calcium and vitamin D compliance. Participants will be specifically asked about denosumab-related toxicities, including skin rashes, infections, perioral numbness, tingling in fingers, or groin or thigh pain indicative of atypical fracture.
- An oral examination will be done at each dose administration that will include a visual examination of the oral cavity, including teeth, mucosa, and jaws.
- Participant must have a negative urine or serum pregnancy test within 14 days prior to or on the day of each denosumab dose.
- Prior to each dose of denosumab, there will be a blood draw including the following laboratory tests:
  - Chemistry (Na^+, K^+, Cl^-, CO_2, BUN, creatinine, glucose, Ca^{+2}, PO_4, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, AST [SGOT], ALT [SGPT]

Results of clinical blood draw and urine or serum pregnancy test will be reviewed prior to denosumab dosing. Denosumab dose will be held in the case of any of the following results:

- Positive urine or serum pregnancy test
- Total bilirubin $\geq$ 2 x institutional upper limit of normal (ULN)
- AST (SGOT)/ALT (SGPT) $\geq$ 1.5 x institutional ULN
- Creatinine clearance $\leq$ 30 mL/min
- Serum calcium or albumin adjusted $\leq$ 8.0 mg/dL
- Serum magnesium < 1.7 mg/dL
- Serum phosphate < 2 mg/dL

If denosumab is not given due to an abnormal blood lab result or other grade 2 or higher toxicity which is possibly, probably, or definitely related to denosumab, the participant can receive a dose of denosumab the following month if the lab values are corrected or the grade 2 toxicity improves to grade 0 or 1. If abnormal blood lab results or other grade 2 or higher toxicities are present in the following month, then no further denosumab will be administered. If the denosumab is not given due to a positive urine or serum pregnancy test, the participant will be taken off study.

After each denosumab dose administration, the participant will be monitored by clinical observation for 30 minutes after each injection to assess for any acute or anaphylactic reactions, such as rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat.

7.4 Evaluation at Time of Surgery

The participant must undergo risk-reducing surgery during the luteal phase (Days 14-28) of the menstrual cycle after the last dose of denosumab (either Dose #1 or #2, which must be administered within 4 weeks of surgery) or 2-8 weeks after starting calcium/vitamin D supplement (in the no treatment control arm). This will allow for some flexibility in the surgical scheduling.

For women with irregular periods or menstrual cycles of >28 days, then the luteal phase will be estimated as the second half of the menstrual cycle.

For women who do not cycle, the date of last menstrual period (LMP) will be documented and the follicular and luteal phases of the participant's menstrual cycle will be estimated based on a 28-day cycle (follicular phase as Days 1-13 and luteal phase as Days 14-28). If randomized to the treatment group, surgery will be scheduled 2-4 weeks after dose 1 or 2 of denosumab as this would be the estimated time frame for the luteal phase. If randomized to the no treatment group, surgery will be scheduled during the estimated luteal phase 2-8 weeks after initiation of calcium and vitamin D supplements. If the participant resumes menstruating during the course of the intervention, the timing of surgery can be rescheduled based on the participant's new LMP.

For women who take hormonal contraceptive with continuous progesterone (*i.e.* progestin IUD), the surgery does not need to be timed with menses.

Given the prolonged absorption of denosumab with drug tissue levels lasting for 3-6 months, a delay in surgery should not affect our biomarker endpoints, however, we will adjust for time (in days) from denosumab treatment (or no treatment) to surgery in our data analysis. The average wait time for risk-reducing surgery at all the participating sites is 2-6 weeks, which is within the timeframe between drug administration and surgery for this trial. If the surgery cannot be performed within the specified time frame of Dose #1 of denosumab, the participant may receive an additional dose of denosumab.

On the day of surgery and prior to the operation, the following procedures must be completed:

- Research blood collection
  - Serum progesterone
  - Serum estradiol
  - Serum denosumab level
  - Serum CTX level
- Clinical blood collection, which should be drawn within 3 days of the day of surgery (-3 days). These may also be drawn at the same time as the research blood collection.
  - Serum calcium level
  - Serum phosphate level
- A focused physical examination based on symptomatology and including vital signs and weight will be obtained. The physical exam may be performed up to 5 days prior to the day of surgery (-5 days).
- An oral examination will be done that will include a visual examination of the oral cavity, including teeth, mucosa, and jaws. If the oral examination is not performed on the day of surgery, it must be performed within 4 weeks of the surgery.
- Concomitant medications will also be obtained. This may also be done via telephone contact the day before the scheduled surgery.
- Toxicity assessment will be performed during a clinical encounter by study personnel. For toxicity assessment, the participant will be specifically asked about denosumab-related toxicities, including skin rashes, infections, perioral numbness, tingling in fingers, or groin or thigh pain indicative of atypical fracture. This may also be done via telephone contact the day before the scheduled surgery.

- Compliance with calcium and vitamin D supplements will be assessed. This may also be done via telephone contact the day before the scheduled surgery.

At the time of surgery, research tissue (fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium [if also undergoing hysterectomy, ~20% or N=12]) will be collected. Immediate fixation in formalin, embedding, processing, and sectioning will follow. Official local pathology review will rule out malignancy. Formalin-fixed paraffin-embedded (FFPE) tissue slides will be sent to Columbia University Irving Medical Center (CUIMC) to evaluate for the following tissue biomarkers:

- Ki67 (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- Apoptosis with cleaved caspase-3 (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- RANK/RANKL expression (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- ER/PR expression (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- CD44 and p53 expression (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- STAT3/pSTAT3 expression (IHC) - fimbrial end of the fallopian tube, ovarian surface epithelium, endometrium (if also undergoing hysterectomy)
- Gene expression profiling - fimbrial end of the fallopian tube, ovarian surface epithelium

7.5 Evaluations During Follow-up Period

Compliance to calcium/vitamin D pill supplements will be conducted at 1, 2, 3, 4, and 5 months (+/- 7 days) after start of treatment (by telephone contact or in person). The participants will be reminded at Month 5 to bring in the pill bottles and remaining pills to their next visit at Month 6.

Seven to 14 days after surgery, calcium and vitamin D supplement compliance will be assessed by telephone contact.

At 6 months (+/- 90 days) after the start of treatment, participant will present for clinic visit for toxicity assessment, calcium and vitamin D pill compliance, and research blood draw for serum CTX level. Toxicity assessment will be performed by research personnel. The pill bottles and remaining pills will be collected at Month 6.

At 12 months (+/- 90 days) after the start of treatment, participant will present for clinic visits for toxicity assessment and research blood draw for serum CTX level. Toxicity assessment will be performed by research personnel.

For toxicity assessment, the participant will be specifically asked about denosumab-related toxicities, including skin rashes, infections, perioral numbness, tingling in fingers, or groin or thigh pain indicative of atypical fracture. The participant will also be assessed for symptoms of hypercalcemia, including stomach upset, nausea, vomiting, headache and decreased alertness.

Tobacco and Alcohol use assessment will be performed at Month 12, using the Month 12 questionnaires (see Appendix C "Alcohol and Tobacco Use Assessment Questionnaires – Month 12").

7.6 Methods for Clinical Procedures

Oral Examination: A visual examination of the oral cavity, including teeth, mucosa, and jaws, will be conducted by the investigator, or designated licensed healthcare professional, at screening, to establish baseline oral health conditions, and at follow-up, to identify and document any new abnormalities or changes in pre-existing conditions. If any new abnormalities

or changes in pre-existing conditions are identified, the investigator may refer the participant to follow up with a dentist or other oral health specialist.

All tissue specimens obtained at the time of surgery will be submitted to the Pathology Department at each site. After standard of care local pathology review to rule out malignancy, residual tissue from the fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium from hysterectomy will be submitted for research purposes. FFPE tissue from 15 immunoblanks (for IHC) and 10 regular blanks (for gene expression profiling) from each research tissue (fimbrial end of the fallopian tube, ovarian surface epithelium) and 15 immunoblanks (for IHC) from the endometrium (if also undergoing hysterectomy) will be shipped to Columbia University Irving Medical Center [CUIMC] as a one-time shipment at the end of the trial (please refer to the Manual of Operations and Procedures for complete instructions). Immunoblanks are coated with a special adhesive for immunohistochemistry analysis, whereas regular blanks are uncoated and will be used for RNA extraction for the gene expression profiling. All tissue slides from the same site will be cut as a single batch to minimize variability in tissue biomarker analyses. For the purposes of the interim biomarker analysis, 2 immunoblanks from the fimbrial end of the fallopian tube will be submitted for the first 10 patients enrolled on the study (for Ki67 IHC evaluation).

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint of the trial is to evaluate the Ki67 proliferation index in fallopian tube fimbrial epithelial cells after denosumab treatment compared to no treatment. The primary biomarker therefore is Ki67, which will be assessed using immunohistochemistry (IHC). Quantitative measures of the expression of Ki67 based upon percentage of positive cells will be scored by a pathologist blinded to treatment assignment.

8.2 Secondary Endpoints

Secondary endpoints include:

8.2.1 Evaluation of proliferation index in ovarian surface epithelium and endometrium (if also undergoing hysterectomy) after exposure to denosumab compared to no treatment using Ki67 as assessed by IHC.

8.2.2 Investigation of other tissue-based biomarkers in the fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium (if also undergoing hysterectomy) after exposure to denosumab compared to no treatment, including:

- Apoptosis with cleaved caspase-3 (IHC)
- RANK/RANKL (IHC)
- ER/PR (IHC)
- CD44 and p53 (IHC)
- STAT3 and pSTAT3 (IHC)

8.2.3 Analysis of gene expression profiling of RANK, cell proliferation, cell cycle progression, and inflammation pathways in the fimbrial end of the fallopian tube and ovarian surface epithelium after exposure to denosumab compared to no treatment

8.2.4 Investigation of serum biomarkers at baseline (pre-treatment) and time of surgery (post-treatment) after exposure to denosumab compared to no treatment, including:

- Progesterone
- Estradiol

- Denosumab drug levels

8.2.5 Investigation of serial serum C-terminal telopeptide (CTX) levels at the following time points after exposure to denosumab compared to no treatment:
- Baseline (pre-treatment)
- Time of surgery
- 6 months after start of intervention
- 12 months after start of intervention

8.2.6 Toxicity profile and frequency of adverse effects in premenopausal *BRCA1/2* mutation carriers undergoing risk-reducing surgery receiving denosumab compared to women receiving no treatment

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), inadequate agent supply, noncompliance, concomitant medications or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE (serious adverse event), lost to follow-up, non-compliance, medical contraindication, withdraw consent, did not undergo surgery within specified time period, or death. Participants found to be ineligible during the screening phase and after signing the Informed Consent document and assigning the PID number, will be considered "screen failures". Such participants will be taken off study and the appropriate end of study CRFs will be completed for these participants. For those participants who need to go off-study due to inability to go to surgery, additional participants will be enrolled to replace these women.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

We will collect and process paraffin-embedded tissue and serum for evaluation of biomarkers.

9.1 Rationale for Methodology Selection

Tissue biomarkers of proliferation and apoptosis (Ki67and cleaved caspase-3), RANK/RANKL, ER/PR, CD44, p53, STAT3, and pSTAT3
Immunohistochemical analysis of the following proteins will be performed on paraffin-embedded sections of fimbrial, ovarian, and endometrial (if also undergoing hysterectomy) tissue by Dr. H. Hibshoosh at the Herbert Irving Comprehensive Cancer Center (HICCC) Molecular Pathology Shared Resource (MSPR) at Columbia University Medical Center (CUIMC), as described in previous reports: Ki67, cleaved caspase-3, RANK/RANKL, ER, PR, CD44, p53, STAT3, and pSTAT3. Qualitative changes and quantitative analysis of the expression of markers will be assessed in a blinded fashion. For each protein, both percentage of positive cells and intensity of staining will be scored by a designated gynecologic pathologist at CUIMC, Dr. X. Chen.

Previous studies investigating the expression of Ki67, cleaved caspase-3, RANK/RANKL, ER, and PR in benign breast

tissue have used IHC-based procedures. Previous studies investigating the expression of CD44, p53, STAT3, and pSTAT3 in the fimbrial and ovarian epithelium tissue have used IHC-based procedures. IHC allows a more precise analysis of protein expression in specific cell types, while avoiding contamination of surrounding connective tissue elements. In addition, if these proteins are to be developed as potential biomarkers, IHC-based procedures, as compared with western blot analysis, provide more feasible methods for analyzing these proteins in clinical specimens.

Gene expression profiling of cell proliferation, cell cycle progression, and inflammation pathways
Gene expression profiling will be performed with the nCounter™ PanCancer Pathways Panel by nanoString Technologies on fimbrial and ovarian tissue of participants from both the treatment and control arms. The system can directly assay tissue to analyze 770 essential genes representing 13 canonical pathways, including cell cycle, apoptosis, and DNA damage control pathways.

This assay can use FFPE-derived and purified total RNA and does not require reverse transcriptase reactions. Results of this assay using FFPE-derived tissue were highly correlated with purified total RNA from fresh tissue (R^2>0.97). The assay demonstrates a high level of sensitivity and precision even at very low levels of expression.

Serum progesterone and estradiol level
Serum progesterone level will be measured at the CUIMC Irving Institute for Clinical and Translational Research Biomarkers Core Laboratory using a LCMS assay on Xevo TQ instrument (Waters; Milford, MA), with an inter-assay and intra-assay precision of <10% and <5%, respectively. Serum estradiol level will be measured using a LCMS/MS method on Xevo TQS instrument (Waters; Mildford, MA), with an inter-assay and intra-assay precision of <10%.

Serum denosumab level
The assay for denosumab drug levels will be performed by the PPD testing facility using the assay developed by Amgen.

Serum C-telopeptide (CTX) level
Serum CTX level will be measured at the CUIMC Irving Institute Biomarkers Core Laboratory. Serum CTX will be measured by ELISA (Immunodiagnostic Systems; Scottsdale, AZ), with an inter-assay and intra-assay precision of 10.9% and 3.0%, respectively. Serum calcium and free calcium will be measured using colorimetric assay with Cobas Integra 400 Plus (Roche Diagnostics; Indianapolis, IN), with an inter-assay and intra-assay precision of 3.5% and 0.99%, respectively.

Please refer to the Pharmacokinetic and Biomarker Methods Development Report for methodology.

9.2 Comparable Methods

We will use established and validated assays for determination of changes in biomarkers expression in this study. We plan to evaluate biomarkers in serum and gynecologic tissue for secondary endpoints evaluation. As to the primary endpoint, the method has been validated and already described in peer-reviewed publications.[49,50]

For secondary serum biomarkers we will utilize standardized protocols of commercially available kits already described in peer-reviewed publications. Standard control samples as well as an in-house pooled serum sample will be analyzed in each run to monitor for inter- and intra-assay coefficient of variation. We will pair samples obtained from the same participant (pre and post-treatment) and run samples in batches to reduce analytical variability.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

A pathologist from each site will perform local review of tissue specimens obtained at the time of surgery. These pathologists are: Dr. Xiaowei Chen (CUIMC), Dr. Lora Ellenson (Cornell), Dr. Chris Crum (DFCI), and Dr. Iris Barshack and Dr. Dov Hershkowitz (Tel Aviv Sourasky).

The tissue-based biomarker analyses of the research tissue will be conducted by the Molecular Pathology Shared Resource (MSPR) for the Herbert Irving Comprehensive Cancer Center (HICCC) directed by Dr. H. Hibshoosh (CUIMC).

The serum biomarkers will be assayed at the Irving Institute for Clinical and Translational Research Biomarkers Core Laboratory directed by Dr. R. Nandakumar (CUIMC).

10.2 Collection and Handling Procedures

For each participant, research tissue (fimbrial epithelium, ovarian surface epithelium, and endometrium [if also undergoing hysterectomy]) will be obtained at the time of surgery. Official local pathology review will rule out malignancy. The protocol for Sectioning and Extensively Examining the FIMbriated End (SEE-FIM) of the fallopian tube will be followed, which includes amputation of each fimbria at the infundibulum, longitudinal sectioning of the fimbria, and cross-sectioning of the remaining fallopian tube at 2-mm intervals.[66] If malignancy, including serous tubal intraepithelial carcinoma (STIC), is detected in the pathology review, this will be reported to the study team. The paraffin section from each tissue type (fimbrial epithelium, ovarian surface epithelium, and endometrium [if also undergoing hysterectomy]) will yield fifteen 4 μm immunoblanks for immunohistochemical analyses. In addition, ten regular 10 μm blanks for gene expression profiling of fimbrial and ovarian surface tissue will be obtained. Blood collected for biomarker analysis at baseline and at time of surgery will be collected in three 4 mL SST tubes. Blood collected for serum CTX level at 6 and 12 months will be collected in one 4 mL SST tube. Detailed instructions on collection and processing for circulating biomarkers are in the lab manual for the study.

For the purposes of the interim biomarker analysis, 2 immunoblanks from the fimbrial end of the fallopian tube will be submitted for the first 10 patients enrolled on the study (for Ki67 IHC evaluation).

10.2.1 Paraffin Sections

- Amount to be collected at the end of the study: 25 unstained slides (15 immunoblanks and 10 regular blanks) of each (fimbrial epithelium, ovarian surface epithelium) and 15 immunoblanks from endometrium (if also undergoing hysterectomy)
- When specimen should be obtained – at time of risk reducing surgery
- Processing of specimen – immediate fixation in formalin, embedding, processing and sectioning to follow
- Labeling of specimen – protocol number (MDA2017-09-03), participant identifier, date of collection
- Tracking of specimens – logs or tracking sheets for participants
- Temperature shipping requirements – room temperature
- Temperature storage requirements – -80°C
- Storage duration – several years

10.2.2 Blood Samples

- Amount to be collected – 10 ml/sample for research blood draw at baseline and time of surgery; 4 ml/sample for research blood draw at 6 and 12 months; 5-20 ml for clinical blood draws at baseline, denosumab injections, and at the time of surgery
- When specimen should be obtained – baseline (progesterone, estradiol, denosumab level, and CTX level), time of surgery (progesterone, estradiol, denosumab level, and CTX level), 6 months (CTX level), 12 months (CTX level)
- Processing of specimen – spun, aliquotted into Cryovials (1 mL each), and promptly snap frozen; any extra serum should be aliquotted in a separate Cryovial; detailed instructions are in the lab manual for the study
- Labeling of specimen – detailed instructions are in the lab manual for the study
- Tracking of specimens – logs or tracking sheets for participants

- Temperature shipping requirements – dry ice
- Temperature storage requirements – -80°C freezer (stored locally and then shipped quarterly to CUIMC)
- Storage duration – several years

10.3 Shipping Instructions

FFPE tissue slides from the surgical specimens from all study sites will be sent to Kristina Hosi at Columbia University, 161 Ft. Washington Ave, Mezzanine level, New York, NY 10032; telephone (212) 304-5580; email: kkh2130@cumc.columbia.edu. Specimens should be sent as a single batch shipment at the end of the study. For the interim analysis, specimens from the first 10 patients enrolled on the study will be sent to the same address when requested by the study team.

Serum specimens (for progesterone, estradiol, denosumab level, and CTX level) will be shipped quarterly on dry ice to Dr. Regina Santella at Columbia University Biomarkers Core Facility, 650 West 168th Street, Room 1608, New York, NY 10032; telephone (212) 305-8158 where further sample processing will take place. The Columbia University Biomarkers Core Facility will serve as a central repository for blood storage.

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.4 Tissue Banking

The Tumor Bank Service is responsible for procurement, storage, retrieval, and distribution of human tumor and normal tissue samples to all investigators at CUIMC. This service protects against the loss of valuable tissue specimens by monitoring of surgical schedules and provides maximum protection to the rights of participants through the acquisition of only those tissues which remain after the appropriate diagnostic and prognostic studies have been carried out in the pathology departments. The tumor bank operates within the scope of a specific protocol that has been approved by the IRB of CUIMC. Tissue collection is done in a manner that ensures optimum quality of tissue without compromising the quality of care and the privacy of the participant's medical information. Participant identifiers are removed from the specimens prior to release to investigators unless they have specific authorization from the IRB to obtain participant identifiers.

Storage: Long term storage is in one of two −80°C freezers that are dedicated to the tumor bank. These freezers are housed in approximately 200 square feet of an equipment room on the 14th floor of the Physicians and Surgeons building in the Pathology Department. These freezers plug into outlets that are connected to the emergency back up generators of the medical center. In case of freezer breakdown, a back up system consisting of liquid carbon dioxide tanks and an automatic dial-out alarm system is activated for protection of samples. Currently, the two freezers are filled at approximately 80% capacity. Additional −80°C freezer space is available nearby on a limited basis only.

Data storage and retrieval: Information on each specimen is organized in spreadsheet form in the Tumor Bank Information System (TBIS). Every case is given a unique tumor bank accession number that is separate from the participant's clinical identifiers. The TBIS also contains important clinical information including tissue type, pathologic diagnosis including tumor subtype, and occasionally, additional clinical information relevant to the case. The exact location of each specimen *i.e.,* freezer shelf, rack, and box number, and number of pieces per case are also recorded.

Quality assurance: Internal quality control experiments are performed in an ongoing fashion to verify the integrity of the specimens in the bank. This includes microscopic examination (by the tumor bank director) of frozen sections to verify the tissue diagnosis, and total RNA extraction and gel electrophoresis (by the research assistant) of selected samples to determine the integrity of the ribosomal RNA bands. These experiments show that approximately 65% of the samples in the bank yield high quality total RNA with either no degradation or minimal degradation visible as a faint smear below well defined bands on an agarose gel. The remainder of specimens yield partially degraded RNA that nevertheless retains utility

for numerous applications. A recently conducted survey of the tumor bank users indicates that tissue has been successfully used in a variety of molecular biological experiments that include RNA, DNA and protein extraction, RT-PCR, Northern blots, gene expression profiling on microarrays, tissue *in situ* hybridization, immunostains, western blots, and genomic sequencing. The vast majority of these users indicate that 75-100% of samples obtained from the tumor bank yielded acceptable results in their particular application.

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed through the toxicity assessment on the day of surgery must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.1.2 All AEs related to surgery that occur during surgery or prior to discharge will not be recorded on the AE CRF (paper and/or electronic). All other AEs, whether or not related to study agent, will be recorded as outlined in the study schedule of events.

11.1.1.3 All SAEs, including all hospitalizations, during the study period will be reported as per DCP SAE reporting procedures, with the following exception:

Hospitalization for planned surgery will not be reported. However, if this hospitalization event lasts longer than the usual period at the institution (as determined by the Site Principal Investigator), it will be reportable as an SAE. Adverse events (AEs) relevant to the prolongation of this hospitalization will be collected and reported on AE CRFs.

Pregnancy/Lactation Notifications will be sent to Amgen (Fax 888-814-8653) within 10 calendar days. The Pregnancy Notification Worksheet and Lactation Notification Worksheet are included in the Appendix F.

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs
The organization that experiences the serious adverse event (SAE) should report the SAE to the following three (3) entities: 1) NCI DCP; 2) DCP's regulatory contractor CCSA; and 3) MDACC, the CLO. Detailed reporting instructions are provided below. In addition, all participating organizations will follow Central IRB (CIRB) and their local IRB requirements for SAE reporting.

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Edward Sauter, MD, PhD
Breast and Gynecologic Cancer Research Group
Division of Cancer Prevention
9609 Medical Center Dr., Rm 5E326
Bethesda, MD 20892-9783
Office: (240)276-7657
Cell: (240)944-3279
Email: edward.sauter@nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug

11.2.2.3 Contact the Consortium Lead Organization (CLO) PI, Dr. Powel Brown, or designee by phone or email listed on the protocol face page within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to the CLO PI or designee via email, phone or fax within 24 hours of knowledge of the event.

11.2.2.4 The Lead Organization and all Participating Organizations will email written SAE reports to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable PDF SAE Report Form.

11.2.2.5 The CLO PI, Dr. Brown, or designee must be copied on the email sent to DCP's Regulatory Contractor CCS Associates, Inc.

11.2.2.6 The DCP Medical Monitor and CCSA regulatory and safety staff will determine which SAEs require FDA submission as IND safety reports. SAEs will be sent to Amgen (Fax 888-814-8653 or secure email svc-ags-in-us@amgen.com) at time of regulatory submission to the FDA.

11.2.2.7 The Site will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs related to the study agent will be followed until resolved.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and subject to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for contract HHSN261201200034I.

12.2 Case Report Forms

Participant data will be collected using protocol-specific CRF developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the DMI application. Site staff will enter data into the e-CRFs in DMI. CRF amendments, if needed, will be submitted to the DCP Protocol Information Office for review and approval prior to deployment in DMI. Approved changes will be programmed into the DMI database by the Consortium Biostatistics and Data Management Core.

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

This study will be monitored on an annual basis by the MD Anderson Cancer Center (MDACC) Data and Safety Monitoring Board, the data and safety monitoring board of record for this study. The Data Safety and Monitoring Board (DSMB) reports to the President, or his designee, as the on-campus representative of MDACC. It oversees the data and patient safety issues for randomized clinical trials that originate at MDACC; that are coordinated or analyzed by MDACC and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;

(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;

(c) Review interim toxicity data and efficacy of treatment;

(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by DCP, NCI, used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as Collaborator(s)) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator@ contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of

the NCI. If a patient participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

12.7.2 For a clinical protocol where there is an Investigational Agent used in combination with (an) other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-party Data").

12.7.3 NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

12.7.4 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

12.7.5 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

12.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

12.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

12.7.8 Any manuscripts reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to DCP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to DCP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a randomized pilot study. A total of 60 participants will be randomized 1:1 to one of the two arms, 1) 1-2 doses of 120 mg denosumab subcutaneously, with Dose #1 given within first 3 days of the menstrual cycle and a subsequent possible dose given in 4 weeks or 2) no treatment. Participants will return for risk-reducing salpingo-oophorectomy with or without hysterectomy during the luteal phase of the menstrual cycle (Days 14-28). In the treatment arm, it will be during the luteal phase of the menstrual cycle after the last dose of denosumab. In the no treatment arm, it will be during the luteal phase of the menstrual cycle within 2-8 weeks of starting calcium and vitamin D supplements. For women who take hormonal

contraceptive with continuous progesterone (*i.e.* progestin IUD), the surgery does not need to be timed with menses. At the time of surgery, research blood draw and tissue collection will be performed. Assuming a 10% drop-out or unevaluable rate, we expect to have 54 evaluable women (27 per arm). With a total number of 54 subjects and assuming a conservative estimation of the standard deviation (SD) of 5.0% in Ki67 expression in fallopian tube epithelium (FTE), we will have 82% power to detect a 4.0% difference (or effect size of 0.8) in Ki67 proliferation index between the denosumab and no treatment groups by applying a two-sided 2-sample t-test at a 0.05 significance level. The sample size calculation is performed using nQuery + nTerim 4.0.

13.2 Randomization/Stratification

In order to keep at minimum the imbalance in treatments, a stratified randomization technique will be applied considering the most relevant prognostic factors, *BRCA1/BRCA2* mutation status and use of hormonal contraceptives within 3 months of registration. We will stratify the randomization by *BRCA* mutation status (*BRCA1* vs. *BRCA2)* and use of hormonal contraceptives within the past 3 months (Yes/No) using the random permutated block design with the block sizes of 2-4. On the designated case report forms, we will also document prior hormonal contraceptive use, duration of use, date of last use, and prior bilateral tubal ligation. If the participant had a recent pregnancy within the past 6 months, we will also document date of pregnancy termination, date of delivery, and date of weaning from breast-feeding (if applicable).

13.3 Accrual and Feasibility

We plan to randomize 60 eligible women (30 per arm) over 16 months at an accrual rate of 3-4 participants per month across all five sites. Assuming a 10% drop-out or unevaluable rate, we expect to have 54 evaluable participants (27 per arm).

13.4 Primary Objective, Endpoint(s), Analysis Plan

Primary endpoint
The primary objective of this study is to evaluate Ki67 proliferation index (IHC) in the fimbrial epithelial cells in the denosumab arm compared to no treatment arm based on the intent-to-treat principle. The primary endpoint is Ki67 expression (IHC) in the fimbrial specimen obtained at the time of risk-reducing surgery. The intent-to-treat population is defined as all participants who are randomized and have Ki67 expression (IHC) measured in the fimbrial specimen after treatment. The primary analysis will be performed per randomization, regardless of the compliance of each participant.

Analysis plan
We will present mean, standard deviation, median and range of Ki67 expression at the time of surgery, by arms, regardless of compliance. In order to evaluate the difference of Ki67 expression between the two arms, 2-sample t-test will be considered. The data will be tested for all underlying assumptions (*e.g.,* normality, homoscedasticity) of the model to ensure that they are all met. In case there is a violation of any of these assumptions, data transformation (log, square-root, etc.) or non-parametric tests such as Wilcoxon rank sum test may be considered. A linear regression may be considered to evaluate the difference of Ki-67 between the two groups, adjusting for other important covariates such as participant age, race, *BRCA* mutation status, etc. The compliance will be included as one of the covariates in the linear regression. A sensitivity analysis will be conducted excluding participants whose surgery did not occur during the luteal phase.

Power analysis
A total of 60 eligible women (30 per arm) will be enrolled and randomized 1:1 to the denosumab or no treatment arms. Assuming a 10% drop-out or unevaluable rate, we expect to have 54 evaluable women (27 per arm). With a total number of 54 subjects and assuming a conservative estimation of the standard deviation (SD) of 5.0% in Ki67 expression in FTE, we will have 82% power to detect a 4.0% difference (or effect size of 0.8) in Ki67 proliferation index between the denosumab and no treatment groups by applying a two-sided 2-sample t-test at a 0.05 significance level. The baseline Ki67 in the fallopian tube epithelium in premenopausal non-*BRCA1/2* mutation carriers is 1.92% with a standard deviation of 2.4%.[53] George *et al*. report that proliferation rate in fallopian tube epithelium does not vary with *BRCA* mutation status[52], while others have reported that *BRCA* mutation carriers have increased proliferation rate in fallopian tube epithelium.[48,49]

We assumed a conservative standard deviation of 5.0% because a standard deviation of 2.4% in Ki67 proliferation index is estimated from normal fallopian tube epithelium.[53]

13.4.1 Supplementary Bayesian Analysis and Interim Futility Monitoring

Based on the same assumptions described above, under the Bayesian framework, we will claim denosumab promising if at the end of the study, there is a posterior probability of more than 97% that the average Ki67 in fallopian tube epithelium (FTE) in subjects treated with denosumab is lower than that in subjects with no treatment, i.e., Prob($\mu_{denosumab}<\mu_{control}|$ data)>97%, where $\mu_{denosumab}$ denotes the average Ki67 in subjects treated with denosumab and $\mu_{control}$ for subjects with no treatment. Per our preliminary data, we make a conservative estimation that the standard deviation (SD) of Ki67 is 5.0%. Under the alternative hypothesis that the mean Ki67 in the denosumab arm is 4.0% lower compared to the mean Ki67 in the no treatment arm, we will have an 82% chance to claim Prob($\mu_{denosumab}<\mu_{control}|$ data)>97% with 27 subjects per arm. For example, the mean KI67 of 10.0% in the no treatment arm vs 6.0% in the denosumab arm corresponds to a 4.0% lower mean Ki67 in the denosumab arm. On the other hand, under the null hypothesis, we will have only 2.8% chance to claim that the denosumab is promising when there is no difference between the two arms. The calculation was performed by simulating 1,000 trials assuming a non-informative Jeffreys prior for Ki67.

Interim futility analysis:
We will implement an interim futility analysis after 10 subjects, 5 in each arm, have been treated and Ki67 been evaluated. We will apply a Bayesian predictive probability method to evaluate whether or not the interim result is promising enough to move forward. We will suspend accrual if the predictive probability of claiming a promising trial is less than 25%, i.e., Prob (Prob($\mu_{denosumab}<\mu_{control}|$ interim data)>97% | future data)<25%. With this rule, we will have more than 70% chance to suspend the trial early if there is actually no difference between the two arms under the null hypothesis. Under the alternative hypothesis, we will have <5% chance to suspend the trial early if the mean Ki67 is 4.0% lower in denosumab arm compared to the mean Ki67 in the no treatment arm. The calculation was done by simulating 100 trials assuming a non-informative Jeffreys prior for Ki67. Upon the suspension of accrual, the interim results and all the study outcomes will be reviewed by the Data Safety and Monitoring Board and experts from the National Cancer Institute to determine whether the study should be stopped early or not. If the interim futility analysis does not trigger the suspension of accrual, the study enrollment will continue.

Bayesian Analysis plan
We will present mean, standard deviation, median and range of Ki67 expression at the time of surgery, by arms, regardless of compliance. In order to evaluate the difference of Ki67 expression between the two arms, Bayesian posterior probability of Prob($\mu_{denosumab}<\mu_{control}|$ data) will be estimated. The data will be tested for normality. In case there is a violation of normality assumptions, data transformation (log, square-root, etc.) will be used before the Bayesian posterior probability is estimated. A Bayesian linear regression may be considered to evaluate the difference of Ki-67 between the two arms, adjusting for other important covariates such as participant age, race, BRCA mutation status, etc. The compliance will be included as one of the covariates in the linear regression. A sensitivity analysis will be conducted excluding participants whose surgery did not occur during the luteal phase.

13.5 Secondary Objectives, Endpoints, Analysis Plans

We will attempt to understand the mechanism of action of denosumab on the primary endpoint of the study. More specifically, we will assess the effects of denosumab compared to no treatment on the secondary endpoint biomarkers.

<u>The secondary objectives are:</u>

- Evaluating Ki67 proliferation index (IHC) in ovarian surface epithelium and endometrium (if also undergoing hysterectomy, N=~12) in the denosumab arm compared to no treatment arm
- Evaluating other tissue-based biomarkers in the fimbrial end of the fallopian tube, ovarian surface epithelium, and endometrium (if also undergoing hysterectomy) in both arms.
    - Apoptosis with cleaved caspase-3 (IHC)

  - RANK/RANKL expression (IHC)
  - ER/PR expression (IHC)
  - CD44 and p53 expression (IHC)
  - STAT3 and pSTAT3 expression (IHC)
- Analyzing gene expression profiling in the fimbrial end of the fallopian tube and ovarian surface epithelium in the denosumab arm compared to the no treatment arm
- Evaluating changes in serum biomarkers at baseline (pre-treatment) and time of surgery (post-treatment):
  - Progesterone
  - Estradiol
  - Denosumab drug levels
- Evaluating serial serum C-terminal telopeptide (CTX) levels at the following time points in both arms:
  - Baseline (pre-treatment)
  - Time of surgery
  - 6 months after start of intervention
  - 12 months after start of intervention
- Monitor safety and adverse effects of denosumab

Analysis plan
All available data will be summarized and analyzed for the secondary analysis. Participant demographic characteristics will be summarized using descriptive statistics. Values of tissue-based and serum biomarkers measurements such as tissue Ki67 proliferation index, serum progesterone, etc., which are continuous variables, will be summarized by descriptive statistics including mean, standard deviation, median and range. Categorical variables, such as adverse events, will be summarized by frequency and proportion. In order to evaluate any change in serum biomarkers from baseline to after intervention between denosumab and no treatment arm, 2-sample t-test may be applied after testing the data for all the underlying assumptions of the model (*i.e.,* normality, homoscedasticity, etc.); in case there is any violation, proper transformation (log, square root, etc.) will be applied to the data. Non-parametric tests such as Wilcoxon rank sum test may be used instead. Furthermore, to investigate the overall changes in serum biomarkers, a linear mixed model that accommodates intra-participant correlation due to repeated measurements will be utilized adjusting for any potential covariates.

For tissue biomarkers, linear regression models will be employed to investigate the association of treatment while adjusting for possible confounders (*i.e.,* age, race, etc.). Normality, homoscedasticity, independence of errors, and lack of multicollinearity in the covariates will be evaluated; if needed, proper transformation will be considered.

For gene expression profiling analysis, nSolver Analysis Software™ (nanoString Technologies, WA) will be used. nSolver performs data normalization using a positive control series comprised of External RNA Control Consortium sequences as well as content normalization probes chosen based on abundance of expression and coefficient of variation to ensure housekeeping genes that display robust expression and minimal inter-sample variability. Geometric mean is used for calculation of normalization factors. Student's t test is used to calculate differential expression.

Other statistical methods may be used when appropriate.

13.6 Reporting and Exclusions

The primary analysis will include all women who undergo surgery and have available Ki67 expression in fimbria specimen. Participants who do not have Ki67 expression in fimbria specimen due to technical failure (*i.e.,* inadequate tissue sample) will be considered unevaluable. All participants will be evaluable for adverse events as long as they receive at least 1 dose of denosumab treatment (active arm only). Participants who do not undergo surgery will be replaced with additional participants enrolled. Every effort will be made to minimize missing data. When it happens, we will build regression models to predict the missing data and apply multiple imputations to fill in the missing data for analysis. Sensitivity analysis will be performed with and without multiple imputations. Statistical inference will be drawn with the caveat of the imputed data.

13.7 Evaluation of Toxicity

All participants who receive study agent will be included in the toxicity evaluation.

The grade, attribution, onset and resolve date of all toxicities will be recorded and summarized for each arm and reported to the DCP monthly and to the MDACC DSMB annually as well as at the end of the study.

13.8 Evaluation of Response

All participants included in the study must be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible.

All of the participants who met the eligibility criteria will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

13.9 Interim Analysis

An interim analysis will be planned after the first 10 participants are enrolled on the trial, as described above in Section 13.4.1.

13.10 Ancillary Studies

No ancillary studies are planned.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

- Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.
- Documentation of training in “Protection of Human Research Subjects” for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

- Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

- Signed Investigator's Brochure/Package Insert acknowledgement form

- Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

- Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:

regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for non-standard of care costs of this study. Study agent will be provided at no cost to the participant. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage. Participants will be compensated $30 for time and travel or the amount needed to cover parking expenses at the time of the first visit during which eligibility will be determined. In the Denosumab group, participants will also be reimbursed $30 for their time and travel or the amount needed to cover parking expenses at the time of each visit with a denosumab injection. In the control group participants will be compensated $30 for time and travel or the amount needed to cover parking expenses at the time of their clinic visit during which vitamin D and calcium will be dispensed.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

Alcohol and Tobacco Use Assessment Questionnaires – Baseline

ALCOHOL ASSESSMENT – BASELINE

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)
   ☐ Refused
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years
   ☐ Refused
   ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

   _____ years of age
   ☐ Refused
   ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

    ☐ Wine
    ☐ Liquor
    ☐ Beer
    ☐ Wine cooler

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

TOBACCO ASSESSMENT – BASELINE

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

- ☐ Yes
- ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

- ☐ Yes In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

- ☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

Signature of Individual Completing This Form_____ Date __ __/__ __/__ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

APPENDIX C

Alcohol and Tobacco Use Assessment Questionnaires – Month 12

ALCOHOL ASSESSMENT – MONTH 12

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____Number of times

☐ None
☐ Do not know/Not sure

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

TOBACCO ASSESSMENT – MONTH 12

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah

- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

☐ Not applicable (This is my first visit to this clinic)

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

APPENDIX D

Resources for tobacco and alcohol quitting

National and local resources to help with alcohol abuse and alcoholism

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them. https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

National and local resources to help with quitting smoking

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Forever Free™ for smokers who have recently quit.
- Forever Free for Baby and Me™, in English and Spanish, for pregnant smokers who have recently quit.
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov Web site.

APPENDIX E

Calcium and Vitamin D3 Tablet Information

INSTRUCTIONS:

1. Please take two 500 mg calcium tablets by mouth daily and one 1000 IU vitamin D3 tablet by mouth daily.
2. Tablets should be taken within the same 12-hour window each day. Tablets not taken within this window are considered missed doses.
3. Please do not take an extra tablet to make up for missed or vomited doses. Take your next tablet as scheduled.
4. Tablets may be taken with or without food or water and may be crushed, chewed, or dissolved in water. Fasting is not required.
5. Please take your tablets at home as scheduled on days where you have a clinic visit for the study.
6. Store the calcium and vitamin D3 tablets at home at room temperature and avoid extreme heat or cold during transportation from the clinic to home.
7. You have been provided with 180 days of calcium and vitamin D3 tablets. Do not discard any tablets or bottles.
8. Bring all bottles and unused tablets to your 6-month study appointment.

Assessment of calcium and vitamin D compliance [67,68]

The following questions should be asked of the participant and the responses documented in the form below.

Date of compliance check (MM-DD-YYYY): _____

Study ID: _____

Circle the compliance timepoint: 1 week 1 month 2 month 3 month 4 month 5 month 6 month

At surgery 1 week post-surgery

1) "In the past week [for 1 week assessment] / month [for all other assessments], how often did you take your calcium and vitamin D supplements as instructed?"

- All of the time (100%)
- Nearly all of the time (90%)
- Most of the time (75%)
- About half the time (50%)
- Less than half the time (<50%)

Questions 2 and 3 are only asked at 1, 2, 3, 4, 5, and 6 month and 1 week post-surgery calls and at time of surgery visit.

2) "In the past month, how often did you forget to take the calcium and vitamin D supplements?"

- Never
- Once in the past month
- 2 to 3 times in the past month
- Once per week
- Several times per week
- Nearly every day

3) "In the past month, how often did you decide to skip the calcium and vitamin D supplements?"

- Never
- Once in the past month
- 2 to 3 times in the past month
- Once per week
- Several times per week
- Nearly every day

Staff Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Staff Name (please print) _____

APPENDIX F

AMGEN Pregnancy Notification Worksheet

AMGEN Lactation Notification Worksheet