An Exploratory Pilot Study of Vitamin D Supplementation in Women with DCIS and/or LCIS

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1.0 Introduction

1.1 Background (17, 22, 23, 24, 34)

Carcinoma in situ is diagnosed in more than 70,000 women annually. Women diagnosed with DCIS and LCIS have a significant risk of developing invasive breast cancer. If DCIS is left untreated 30-40% will develop invasive cancer. LCIS is a risk factor for development of breast cancer; women with LCIS have a 2% risk per year of developing breast cancer (22, 23, 34). While endocrine therapy has been shown to lower this risk, less than a third of patients who could benefit from chemopreventive endocrine therapy actually take it. Additional interventions are needed to prevent invasive breast cancer in women with carcinoma in situ.

1.2 Rationale

Evidence for Vitamin D as a chemopreventive agent (3, 14, 31)

In vitro, calcitriol, the most potent metabolite of vitamin D, inhibits a variety of cellular pathways that promote cell proliferation and survival. Vitamin D has been shown to reduce the growth of breast cancer precursor cells in cell culture studies. In animal models Vitamin D has been shown to prevent the growth and progression of transplanted cell lines MCF710A, which is a model of pre-invasive cancer. Serum Vitamin D level deficiency correlates with an increased risk of breast cancer, and reduced survival of breast cancer patients. Vitamin D is also recognized to have effects on immune cell function and autoimmunity. The safety profile of oral Vitamin D, and its metabolite calcitriol, was well established for moderate term and acute therapy worldwide. Potential additional primary and secondary benefits of vitamin D are a) the suppression of carcinogen-induced transformation or progression of breast epithelium, and b) the enhancement of innate immune defense of pre-invasive breast cancer lesions, and c) its qualification as a combination therapy when combined with other neoadjuvant therapies for DCIS.

Proliferative index, Ki67 as a biologic marker for response in breast cancer (1, 2, 9, 10, 17)

For in situ and invasive carcinoma, Ki67 is an immunohistochemistry stain and a marker of proliferative activity. It is also a component of multiple genomic prognostic profiles including Oncotype Dx. The proliferative index, determined by immunohistochemistry Ki67, correlates with potential for progression from carcinoma in situ to invasive carcinoma. Further, measurements of Ki67 before and after neoadjuvant therapy has been validated as a marker of response to therapy for breast cancer, both invasive carcinoma and carcinoma in situ.

1.3 Role of autophagy and calcium efflux in carcinoma in situ (5-8, 30, 31)

The cell survival pathway autophagy is upregulated in DCIS. Autophagy is required for the survival of human cancer stem-like DCIS cells. Calcium efflux pumping through the PMCA2 channel protects the neoplastic cell from elevated intracellular calcium. PMCA2 is also upregulated in breast cancer cells, notable in those which overexpress her2. Vitamin D elevates intracellular calcium that may be toxic and suppress growth of DCIS stem like cells. We have shown experimentally that vitamin D treatment will reduce the ability of breast cancer cells to engage autophagy for survival.

1.4 Feasibility and Preliminary Studies

High dose Vitamin D repletion regimen (3, 14, 15, 19, 20, 22, 27, 28, 29, 33, 35)

Serum levels of 25(OH)D3, are the best measure of the status of vitamin D repletion in humans. Considerable evidence indicates that a large proportion of men and women have levels of 25(OH)D3 below what many would accept as the lower limit of normal (<32mg/ml). Studies indicate that 60-75% of adults have serum 25(OH) D3 levels <32ng.ml. This is one of the prime observations leading to many studies seeking to determine whether this high frequency of "low" 25(OH) D3 level contributes to the causation and/or prognosis of many types of cancer. While many studies have reported an association between low 25(OH) D3 level and risk and/or prognosis of almost every type of cancer, no study has ever tested the hypothesis: "does restoration of 25(OH)D3 levels into the normal range reduce the incidence or improve the prognosis of any cancer". The Women's Health Initiative is often noted to be a study which evaluated the role of D3 supplementation. While this is true and the WHI was a very well-conducted study of a large number of individuals, and there was no impact of D3 supplementation on cancer incidence, the dose of D3 which was administered (400IU/d) was insufficient to raise the 25(OH)D3 levels in the study participants and was lower than the current dose of D3 supplementation recommended by the IOM (600-800IU/d in adults). Studies demonstrate that in order to increase the 25(OH)D3 serum level into the normal range in >95% of individuals a dose of 4000IU/d must be administered.

Hathcock and colleagues surveyed the world literature in 2007 and found no case of vitamin D intoxication in individuals with a serum 25(OH)D3 level <200ng/ml and/or taking <30,000IU D3 continuously (14). "High dose" D3 administration has repeatedly been shown to be safe: 500,000IU once was administered to nursing home residents by Sanders and colleagues and no vitamin D toxicity was seen (33). Baseline 25(OH) D3 levels were 22ng/ml and increased at 1 month after dosing to approximately 28ng/ml and were approximately 36ng/ml at 3 months. Studies of 4 groups of men (30/group) who received either 4000IU, 6000IU, 8000IU or 10,000IU daily for 6 months. The highest 25(OH) D3 level noted was <140ng/ml and no consistent changes in serum or urine calcium levels were seen.

Schleck and colleagues evaluated 3 dosing regimens to replenish vitamin D stores in 3 months. One hundred and fifty (150) subjects were randomized into three groups, each to receive, orally, a loading dose of 50,000, 100,000 or 200,000 IU of D3 at Week 0, followed by 25,000, 50,000 or 100,000 IU at week 4 and week 8 (33). A significant increase in 25(OH)D3 level was observed at week 12 ($p < 0.0001$) with a mean change from baseline of 7.72 ± 5.08, 13.3 ± 5.88 and 20.12 ± 7.79 ng/mL. A plateau was reached after eight weeks. No related adverse events were recorded. This study demonstrated a linear dose-response relationship with an increase in 25(OH)D3 levels proportional to the dose administered. A loading dose of 200,000 IU VTD3 followed by a monthly dose of 100,000 IU was the best dosing schedule to correct the VTD status in 3 months (33). This study demonstrates the safety of this dosing regimen but also emphasizes the fact that rapid restoration of 25(OH)D3 levels will require a different approach.

We have developed and studied a "rapid D3 repletion" approach based on a pharmacokinetic analysis of our 4000IU, 6000IU, 8000IU and 10,000IU QD repletion trial. This analysis indicated that a 100,000IU loading dose D1 followed by 4000 IU QD will rapidly replete 25(OH)D3 levels as noted below:

As this dosing regimen has been shown to be safe and is pharmacokinetically attractive vis a' vis restoration of 25(OH)D3 levels we will employ this regimen in the experimental arm of this trial.

PINC trial (8)

The PINC trial evaluated the impact of neoadjuvant chloroquine on DCIS. In this study, chloroquine successfully inhibited autophagy and the proliferative index of the DCIS. This study treated patients after diagnosis of DCIS with chloroquine until the surgery. Measurements of autophagy proteins and the proliferative index were evaluated by immunohistochemistry. A control group of untreated patients were included. Chloroquine therapy resulted in decrease in the proliferative index and inhibition of the autophagy pathway proteins (8).

1.5 Neoadjuvant Endocrine Trials in Breast Cancer (9, 10, 11, 23, 24)

The Alliance Neoadjuvant trial compares three endocrine therapies in the neoadjuvant setting. Ki 67 testing was done prior to start of therapy and one month later. Suppression of the ki67 after one month of therapy has been validated as a reliable indicator of response to endocrine therapy. If the ki67 is not adequately suppressed at one month, patients are transitioned to chemotherapy.

Neoadjuvant therapy for ductal carcinoma in situ have included trials with both tamoxifen and aromatase inhibitors. In these trials the proliferative index, ki67, has been measured and accurately correlated with response to therapy. These prior studies also included control patients, to evaluate potential variability in ki67 from the core biopsy to the surgical specimen.

2.0 Proposed Study Overview

Patients, who have been diagnosed by core biopsy with carcinoma in situ, ductal or lobular, will be evaluated for vitamin d supplementation. Patients with vitamin d levels less than 50 will be eligible for participation. They will receive a 28 days (+/- 4 days)) schedule of vitamin D supplementation and then proceed with the standard of care of surgical excision. Immunohistochemistry studies will be performed on the diagnostic core biopsy and the surgical specimen to evaluate the impact of vitamin d supplementation on: the proliferative index-ki67, proliferative marker- PCNA, proteins of the autophagy pathway (LC3B, ATG7), her2 localization, and levels of PMCA2 - calcium efflux channel.

This trial will be the first to study the effect of vitamin D in vivo on the proliferation index and the state of the autophagy pathway in carcinoma in situ lesions.

2.1 Primary objective

This pilot study will explore whether changes in proliferative index, as determined by Ki67, can be identified after 28 days (+/- 4 days) of vitamin D supplementation in patients with ductal carcinoma in situ or lobular carcinoma in situ.

2.2 Secondary objectives

2.2.1 Explore the potential impact of Vitamin D therapy on autophagy associated proteins (LC3B & ATG7) and PCNA proliferation marker.

2.2.2 Test other exploratory measures after Vitamin D supplementation: Calcium transport proteins (PMCA2) and HER 2 localization

3.0 ELIGIBILITY ASSESSMENT AND ENROLLMENT

3.1 Eligibility Criteria

- Subjects must have a tissue diagnosis of lobular carcinoma in situ or ductal carcinoma in situ and being scheduled to undergo excision of their cancer.
- Subjects must be female at least 18 years of age.
- Subjects must have a signed consent
- Normal liver function based on (total bilirubin and AST <1.5 x Upper Limit of Normal).
- Serum creatinine < 2.0 mg/dL
- Serum 25 (OH) D levels < 50 ng/ml
- Calcium within the normal range (8.5-10.2 mg/dL)
- ECOG performance status 0-2.
- Are able to swallow and retain oral medication.
- Subjects should be willing to abstain from use of hormonal therapies (e.g. hormone replacement therapy, oral contraceptive pills, hormone-containing IUDs, and E-string).

3.2 Exclusion Criteria

- Inability to consent.
- Current use of hormone-containing forms of birth control such as implants (i.e. Norplants, or injectable (i.e. Depo-Provera).
- Currently lactating.
- Patients with history of renal or hepatic insufficiency.
- Used an investigational drug within 30 days or 5 half-lives, whichever is longer, preceding the first dose of study medication.
- History of granulomatous disease such as tuberculosis or sarcoidosis
- History of Vitamin D supplementation > 2000 IU/day within the last 2 months.
- History of hypoparathyroidism

3.3 Research Eligibility and Enrollment

Eligible patients will be identified at the participating study site. A screening log will be kept documenting the review of potentially eligible participants as well as reasons for non-enrollment. They may be directly referred from private practice offices and the hospital-based clinics.

Once approved, this study will be listed on the NIH website Clinicaltrials.gov to enable referring physicians and patients to identify participants for referral. While the specific mechanism through which patients are identified may vary slightly, informed consent will be obtained from all patients according to the following procedures for enrollment.

Patients who are pre-screened by the research staff as meeting the eligibility criteria will be invited to participate in the study. After patient signs the consent to the study, the clinical research coordinator (CRC) will complete screening assessments and full eligibility review will be conducted. Patients who meet the study's eligibility criteria will be enrolled by the CRC. The patient will then be assigned a unique study ID number by the research staff.

3.4 Subject Numbering

The Clinical Research Coordinator assigns a unique subject number based on entry order.

4.0 STUDY IMPLEMENTATION

4.1 Overall Study Design

Once consented, the subject will complete screening assessments. If the patient meets the eligibility criteria for the study, the patient will be enrolled and scheduled for treatment Day 1. The clinical research coordinator will complete the request to obtain a tumor block from the patient's recent biopsy. Enrolled patients will receive the Vitamin D supplementation medication for 28 days (+/- 4 days). The patient will continue with the surgical standard of care treatment planning/scheduling process. A tumor block or 12 unstained slides from the patient's breast biopsy and breast surgery will be requested by the CRC and sent to GMU for analysis.

This study utilizes tissue obtained (1) from leftover tissue following core diagnostic biopsies or excisional biopsies and (2) from specimens obtained at the time of standard of care and surgical treatment of breast cancer. Obtaining these samples will not interfere with the histologic pathology diagnosis.

4.2 Initial Intervention

Patients with a diagnosis of lobular carcinoma in situ or ductal carcinoma in situ who fit the eligibility criteria will be enrolled following informed consent.

After the pathologic diagnosis are rendered for (1) and (2) above, including immunohistochemistry markers, diagnostic paraffin blocks will be requested from the appropriate laboratory by the Inova research team. Inova will coordinate transfer of the block or 12 unstained slides to George Mason University. A de-identified pathology report

must be submitted with the patient's tissue block or 12 unstained slides. The de-identified pathology report must have the unique study-assigned patient ID.

Tumor block or slides can be picked-up by an authorized GMU representative or sent to GMU. The address to send the samples is:
Dr. Virginia Espina
Center for Applied Proteomics and Molecular Medicine
George Mason University
10900 University Blvd
Bull Run Hall Room 351
Manassas, VA 20110

4.3 Patient Consent

Informed consent on this protocol permits transfer of anonymous recut tissue sections to George Mason University for analysis of molecular endpoints. See also Section 13 Ethical and Regulatory Considerations.

4.4 Samples will be anonymous

Patients and tissue samples will be assigned a unique patient number to provide patient confidentiality during the molecular analysis at GMU. Clinical investigators will have access to the linkage code for correlative analysis. The protocol scientific investigator(s) handling the samples will be blinded as to the patient identification, patient data, and outcome during the sample analysis. Outcome data will be provided to the clinical research team for correlative and potentially causative analysis.

4.5 Clinical Tissue and Sample Acquisition and Processing

The human therapeutic molecular endpoint target is proliferation index, ki67. The proposed treatment is oral Vitamin D administered for 28 days (+/- 4 days) between the time of diagnostic biopsy and the time of surgical excision. The impact of the therapy on activity state of the proliferative markers (Ki67, PCNA), proteins of the autophagy pathway (LC3B, ATG7), HER 2 localization, and calcium efflux (PMCA2) in at least 500 DCIS or LCIS intraductal cells in 5 ducts are scored under 200x.

4.6 Drug Administration

Patients will receive 100,000IU PO loading dose at Day 1. The loading dose must be administered to the patient at the investigator's site. Patients will be dispensed 10 capsules of 10,000 IU/capsule and the CRC must document administration of the loading dose at site once completed.

Patients will be dispensed 32 capsules/bottle of 1cap/4000 IU PO QD on Day 1 visit to take home. Bottle must be labeled with instructions on how to take the drug and the assigned patient ID number. Patients will be instructed to take 1 capsule per day, with water, from Day 2 to Day 28 (+/- 4 days) using the dispensed study bottle. They will be instructed to stop taking their daily vitamin D3 dose after 28 days (+/- 4 days) of treatment. Patient's confirmation of understanding for the dosing regimen must be documented by the research nurse/coordinator. A study drug diary will be provided at Day 1 visit and

patients will be instructed to complete the study drug diary daily from Day 2 to Day 28 (+/- 4). Patient's report of vitamin-D3 intake from the diary must be reconciled against the number of capsules returned at Day 28 (+/- 4) visit. Patient must be clearly instructed to discontinue daily dose of vitamin D after 28 days (+/- 4 days) of daily therapy.

4.7 Monitoring toxicity

Any unexpected AEs or SAEs will be reported to the appropriate agencies and individuals. Stopping Rules, Off Agent, and Off Study criteria will be in place for any unexpected serious adverse events or protocol variances.

4.8 Compliance

Compliance with medication will be defined as follows. Each subject will be assigned one of the following categories: 1) compliant with medications, (i.e. took pills as directed, assessed at Day 14 (+/- 3), Day 21 (+/- 3), and at end of treatment 2) non-compliant with medication, did not take pills as directed.

4.9 Dose Modification

Subjects who have any Grade 2 toxicity for greater than 1 week duration that is at least possibly related to the study drug will have study drug withheld until toxicity resolves to ≤ Grade 1. Study drug may be reintroduced, but if Grade 2 toxicity re-occurs, study drug(s) will be withheld for two weeks and possibly discontinued. Subjects with Grade 3 or greater toxicity that can at least possibly be attributed to study agent(s) will have the medication discontinued. Under no circumstance will surgery be delayed because of D3 interruption.

4.10 Concomitant Medications

All concomitant medications taken for the past 14 days prior to screening must be documented and reviewed with patient for indication, dose, and history. Patient's concomitant medications must be reviewed at every study visit. If concomitant therapy must be added or changed, the indication, dose and name of the drug/therapy must be documented.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient is allowed. However, certain medications may interact with cholecalciferol. The following medications are contraindicated while on the study:

- Aluminum Hydroxide
- Sucralfate
- Multivitamins / Flouride (wth ADE)
- Danazol (Danocrine)
- Cardiac glycosides: Deslanoside (cedilanin-D), Digitoxin (Crystodigin), Digoxin (Lanoxicaps, Lanoxin
- Thiazide and Thiazide-like Diuretics: Bendroflumethiazide (Naturetin) Benzthiazide (Exna) Chlorothiazide (Diuril, Diurigen) Chlorthalidone (Thalitone, Hygroton) Hydrochlorothiazide (Esidrix, HydroDiuril, Hydro-Par, Oretic) Hydroflumethiazide (Diucardin, Saluron) Indapamide (Lozol) Methyclothiazide (Enduron, Aquatensen)

Metolazone (Zaroxolyn, Diulo) Polythiazide (Renese) Quinethazone (Hydromox) Trichlormethiazide (Metahydrin, Naqua, Diurese)

- Other anticancer agent other than the study medication administered as part of this protocol. If such agents are required for a patient then the patient must be removed from the protocol treatment.

- Other investigational therapy must be given to patients.

- Other hormonal therapies during treatment with study drug.

4.10.1 A review of concurrent medications will also be determined at this time to ensure that a potential subject is not ineligible as per criteria 3.1-3.2.

4.10.2 A directed history will be obtained to ensure that subject is not ineligible for medical reasons as per criteria 3.1-3.2.

- Intake Study Calendar

  A study drug diary will be provided to the patient on Day1. Patient will be instructed to document their daily dose of Vitamin D3 at Day 1, at the investigator's site, and for the next few weeks for a total of 28 days (+/- 4 days). Site will review and ascertain patient's adherence with protocol therapy at Day 14, Day 21 phone call, and at the end of treatment visit. The study drug diary must be filed with the patient's folder and data will be reported on the case report form.

- Surgical Guidelines

  The patients will then undergo appropriate surgical therapy for their cancer. Subjects should have stopped vitamin D3 therapy at the time of surgery. Surgical pathology formalin fixed paraffin embedded (FFPE) block or 12 unstained slides will be requested by the research staff after the final diagnostic report is rendered.

- Remuneration

  Participants will not be paid for enrollment in this study. No funds for Compensation for injury to research subjects are available from The Inova Dwight and Martha Schar Cancer Institute, the study physician practices, George Mason University, or the Federal, State or Local Governments.

5.0 CLINICAL STUDY EVALUATIONS

Schedule of Events for Breast Cancer Vitamin D Trial (Table 1.0)

Study Calendar							
	Screen	Treatment Phase				Surgery	Post Op Visit (within 30 days +/-14 days post-Surgery)
		Day 1 (Pre Dose)	Day 14 (+/- 4 days) [6]	Day 21 (+/- 4 days)	Day 28 (+/- 4 days)		
Written Informed Consent[1]	x						
Subject Demography	x						
Medical History	x						
Disease History	x						
Therapy History	x						
Inclusion/Exclusion Criteria[2]	x						
Safety Assessments							
Concomitant Medication(s)	x						x
Physical Examination	x						x
ECOG status, Vital Signs[3]	x						x
Adverse Events	x	x	x	x	x	x	x
Laboratory Assessments							
Chemistry[5], 25(OH)D3, CBC	x				x[7]		x[8]
Pregnancy Test[4]	U/S[4]						
Tissue Samples							
Recut Tissue/Tissue Block IHC Biomarkers		A				B	
Tissue Sample Histopathologic Assessment		A				B	
Investigational product							
Dispense Invest. Product.		x					
Assess Invest. Product compliance		C	C	C			C

Notes:

1. All screening assessments should be performed within 30 days prior to administration of first dose of study drug, unless otherwise indicated.
2. After all screening evaluations have been completed and the data are obtained, the inclusion and exclusion criteria must be reviewed to confirm subject eligibility.
3. May include height, weight, blood pressure, temperature and heart rate.
4. Pregnancy test: urine = U, serum = S if indicated by reproductive age (< 60 yrs., or lack of confirmed tubal ligation or hysterectomy)
5. Chemistry panel will include: Na, P, Cl, CO2, Glucose, BUN, Creatinine, Calcium, Total Protein, Albumin, Globulin, Bilirubin, AST, ALT, Alkaline Phosphatase, EGFR, CBC with differential. Vitamin D3 levels will be checked at screening and at end of treatment visit.
6. Assessments for Day 14 and Day 21 can be completed via a nurse/coordinator phone call to patients under treatment.
7. Patient must agree to have the vitamin D3 level checked +/- 3 days prior to day of breast surgery.
8. For patients with vitamin D levels above normal at vitamin D3 level check at Day 28 visit, vitamin D3 level must be assessed at the post-surgery visit with activities at post-surgery.

A. Tissue block or 12 unstained slides from the patient's diagnostic biopsy must be requested after patient's confirmation of eligibility and registration to the study. Refer to section 4.0.

B. Tissue block or 12 unstained slides from patient's breast surgery must be requested. Refer to section 4.0.
C. Drug compliance will be assessed via a drug diary provided to the patient at Day 1.

5.1 Baseline Test/Pre-treatment Evaluation

Patients with DCIS or LCIS and who are considered to be eligible will have the following procedures performed at the time the patient is enrolled for the study:

- Medical history and physical exam (including collection of height, weight, ECOG, medication review)
- Laboratory Blood Test including:
  - Serum potassium, Calcium
  - Total bilirubin
  - AST/ALT
  - Creatinine
  - 25(OH)D3 level
  - CBC with differential

5.2 Evaluations during pre-excision supplementation

The following procedures will be done during the patient's vitamin D regimen through a nurse/coordinator phone call at Day 14 and Day 21 of study:

- Compliance to daily drug therapy
- Clinical Assessment and adverse event evaluation will be done at Day 14, Day 21, and on the Post-Op visit on the study.

5.3 Evaluations at completion of pre-excision supplementation

The following procedures will be done following the completion of the patient's supplementation:

- Physical exam (including collection of height, weight, ECOG)
- Laboratory Blood Test including:
  - Serum potassium, Calcium
  - Total bilirubin
  - AST/ALT
  - Creatinine
  - 25(OH)D3 level
  - CBC with differential

- Paraffin block recut tissue or 12 unstained slides will be collected for the study after the definitive diagnosis is rendered.

- Assess Research Study drug compliance

5.4 Post-Surgery Follow-up

- The patient will resume standard of care follow up for the treatment of their DCIS or LCIS.

- Serious adverse event evaluation will be collected for up to 1 month following the patient's surgery.

- For patients with vitamin D levels above normal at the Day 28 (+/-4 days) lab visit, a re-check of vitamin D levels is advised.

6.0 CRITERIA FOR CLINICAL EVALUATION AND ENDPOINT DEFINITION

6.1 Primary Endpoint

Ki-67, is used to establish the proliferation index as a primary endpoint. At least 500 DCIS or LCIS intraductal cells in 5 ducts are scored under 200x. Proliferation index is the number of nuclei labelled versus total nuclei examined.

6.2 Off Study Criteria

Subjects may go "off-study" for the following reasons:

- Subject request
- Non-compliance with protocol, as per clinician's discretion
- Intolerable Grade 2 toxicity or Grade 3 toxicity or greater at least possibly attributable to study medication, as per clinician's discretion
- Taking hormonal therapies during treatment with study drug (e.g. t, hormone replacement therapy, oral contraceptive pills, hormone containing IUDs, E-string)
- Inova Schar Cancer Institute, and/or George Mason University, as Sponsors, may discontinue investigation at any time
- Subject lost to follow up
- Death

7.0 SPECIMEN MANAGEMENT

7.1 Laboratories

The laboratories performing specimen analysis are:

Immunohistochemistry will be performed at George Mason University Center for Applied Proteomics and Molecular Medicine, Manassas, VA 20110, under the direction of Lance Liotta, MD, PhD, and Virginia Espina PhD.

7.2 Tissue Collection and Handling Procedures

7.2.1 Shipping Instructions

Tissue blocks or 12 unstained slides will be transported by FEDEX to George Mason University Center for Applied Proteomics and Molecular Medicine (CAPMM). Samples must be shipped or picked-up by a GMU representative within 30 days of study enrollment.

7.2.2 Tissue Banking

GMU CAPMM personnel will maintain an inventory (electronic and hard copy) of tissue recut sections transferred to GMU. Recut sections will be will be stored at appropriate temperature and condition for the type of analysis to be performed.

8.0 Adverse Events Reporting

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study drug even if the event is not considered to be related to study drug. Study drug includes the drug under evaluation, and any reference or placebo drug given during any phase of the trial.

Medical conditions/disease present before starting study treatment are only considered adverse events if they worsen after starting study drug, including any procedures specified in the protocol. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded. Adverse events include all deaths that occur while a participant is on a study.

A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 11.0, or package insert. Stopping Rules for hypercalcemia, Off Agent, and Off Study criteria will be in place for any unexpected SAEs or protocol variances.

8.1 Adverse Events (AEs)

8.1.1 Reportable AEs

All AEs that occur after the study treatment is started (and baseline symptoms obtained) must be recorded on the AE Case Report Form (CRF) whether or not related to the study agent.

8.1.2 AE Data Elements

- AE reported Date
- AE Verbatim Term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as an SAE
- Action taken with the study agent
- Outcome of the event
- Whether or not patient discontinued due to AE
- Comments

8.2 Severity of AEs

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE 4.0 provides descriptive terminology and a grading scale for each AE listed. A copy of the CTCAE 4.0 can be found at http://ctep.cancer.gov.

AEs will be assessed according to the CTCAE 4.0 grade associated with the AE term as

Grade 1 Mild Adverse Event

- Asymptomatic
- Mild or minor symptoms
- Marginal clinical relevance
- Clinical or diagnostic observations only

- Intervention not indicated
- Non-prescription intervention indicated

Grade 2 Moderate Adverse Event
- Intervention indicated
- Minimal, local, non-invasive intervention (i.e. packing, cautery)
- Limiting instrumental ADL (i.e. shopping, laundry, transportation, ability to conduct finances)

Grade 3 Severe Adverse Event
- Medically significant but not life-threatening
- Inpatient or prolongation of hospitalization indicated
- Important medical event that does not result in hospitalization but may jeopardize the patient or may require intervention either to prevent hospitalization or to prevent the AE from becoming life-threatening or potentially resulting in death or
- Disabling
- Results in persistent or significant disability or incapacity
- Limiting self-care ADL (i.e. getting in and out of bed; dressing; eating; getting around inside; bathing; using the toilet)

Grade 4 Life-threatening Adverse Event
- Life-threatening consequences
- Urgent intervention indicated
- Urgent operative intervention indicated
- Patient is at risk of death at the time of the event if immediate intervention is not undertaken
- Blindness or deafness (need to decide if unilateral or bilateral)

Grade 5 Fatal Adverse Event
- Death

8.3 Assessment of relationship of AE to treatment

The possibility that the AE is related to study drug will be classified as one of the following: unrelated, unlikely, possible, probable, and definite as described below:

Unrelated There is no evidence of causal relationship.

Unlikely There is little evidence to suggest that there is a causal relationship (i.e. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (i.e. the subject's clinical condition, other concomitant treatments).

Possible There is some evidence to suggest a causal relationship (i.e. the event occurred within a reasonable time after administration of the trial medication). The influence of other factors may have contributed to the event (i.e. the subject's clinical condition, other concomitant events).

Probable There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be eliminated.

8.4 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. No AEs will be collected post-surgery, only those events determined to be SAEs will be reported up to 1 month post-surgery.

8.5 Serious Adverse Events (SAEs)

8.5.1 Definition

ICH Guideline E2A and Fed. Reg. 62, Oct 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (The term life-threatening refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner

8.5.2 Reporting Responsibility

Each serious adverse event (but not pregnancies) must be reported by the investigator to the Clinical Investigator or his/her delegate within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related.

Information to be reported includes:

- Date and time of the SAE
- Date and time of the SAE report
- Name of the reporter
- Call back phone number
- Institution conducting the study
- Description of the SAE, including attribution to drug and expectedness

Follow-up information about a previously reported serious adverse event must also be reported to the Clinical Investigator within 24 hours of receiving it. If the serious adverse event has not been previously documented (new occurrence), and it is thought to be related to a study drug (or therapy), the Principal Investigator may contact the investigator to obtain further information. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with Vitamin D that this serious adverse event has been reported.

8.5.3 Reporting Procedures

The investigator must complete the FDA MedWatch 3500a form to assess the relationship to study treatment and send the initial completed MedWatch form and CRF SAE coversheet by fax within 48 hours to the Medical Safety Monitor. The investigator must then ensure that the form and coversheet are accurately and fully completed with follow-up information and fax those to Medical Safety Monitor

within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events. The original and the duplicate copies of the FDA MedWatch form, CRF SAE coversheet, and the fax confirmation sheet must be kept with the case report forms at the study site.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The MedWatch form, CRF SAE coversheet, and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

9.0 Study Monitoring

9.1 Human Subjects Protection

9.1.1 Rationale for subject selections

This study will be open to all women who meet the study's inclusion and exclusion criteria. Efforts will be made to extend accrual to all minority patients, but it may be difficult to obtain representatives from all minority racial and ethnic groups. For safety reasons, pregnant and lactating women and children are excluded from this study. Men will be excluded to eliminate possible confounding hormonal factors.

9.1.2 Evaluation of benefits and risks/discomforts

Benefits include the potential of reducing the risk of developing invasive breast cancer, recurrence or metastasis. Potential risks include medication side effects.

9.1.3 Patient Withdrawal

The patient will be removed from the study for the following events:

- Patient request for any reason.
- Inadequate tissue for pathologic diagnosis or inadequate tissue to spare recut sections.
- Non-compliance with study protocol and/or medications.
- If a patient wishes to withdraw from the study at any time, the patient or their physician will contact the CRC or an Investigator, who will initiate the withdrawal process. When the patient withdraws from the study, their samples will be destroyed or returned to pathology if they have not been processed at GMU. The patient will be unable to retrieve the specimen beyond this point. Any samples remaining at GMU will be destroyed as requested. Confirmation of destruction of the sample will be forwarded to the physician and the PI. Once the patient withdraws, no further follow-up or contact will be pursued.

10.0 Data Management

This study will report clinical data using REDCAP database. Data will be prospectively collected and uploaded into the coordinating site database at least once a month. Subsequently, the information uploaded into the database will be systematically checked by Data Management staff. Obvious errors will be corrected by the study personnel. Errors or omissions will be documented and maintained within the patient files.

10.1 Case Report Forms

Participant Data will be collected using protocol specific clinical research forms tailored to this study. Study staff will enter data into the CRF on-line according to pre-established sponsor standards and procedures. Amended CRFs will be submitted to the Study PI for review and approval. Approved changes will be programmed into the study database.

10.2 Source Documents

All Source documents will be maintained by the clinical research staff at investigational site. Patient research charts or electronic medical records containing the source documents include laboratory records for verification of eligibility and data to confirm molecular classification as well as other data will be entered onto the CRFs. Medical records are accessed by the IRB-approved research investigators and staff only for the purposes of completing the study patient information sheets.

A copy of the signed informed consent will be in the patient's medical record and the original will be held in the research office. A copy of the signed informed consent will also be given to the patient.

10.3 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) and IRB guidance requirements. The records will be retained for at least three years after completion of the research. The records will be accessible for inspection and copying by authorized persons.

10.4 Confidentiality of Patient Data

The clinical site is responsible for the confidentiality of the data associated with patients registered in this study in the same manner, as it is responsible for the confidentiality of any patient data within its sphere of responsibility. For patients registered to this study, there are additional considerations related to the necessity of sharing of research data with George Mason University. The patient grants permission to share research data with study research staff at George Mason University in the consent document.

10.5 Clinical Trials Agreement

This study will be performed under the auspices of the overarching GMU /Inova Clinical Trials Agreement.

10.6 Data and Safety Monitoring Plan

A Data Safety Monitoring Plan is formed to assure subject safety in this clinical trial. Clinically, this is a single institutional study using agents that are well researched with known side effect profiles. All SAEs will be coded using MedDRA version 12.0 for reporting to the FDA, the medical monitor and the IRBs as required. Oversight of the conduct, quantitative recruitment, compliance progress and administrative structure will be provided by Inova IRB Committee. The committee will submit written recommendations on the progress of the study to the study Principal Investigator.

10.7 Data Collection Schedule

The schedule of data collection for this study is provided in Table 2.

Table 2.0. Data collection schedule

Study Calendar							
	Screen	Treatment Phase				Surgery	Post Op Visit (within 30 days +/- 14 days post Surgery)
		Day 1 (Pre Dose)	Day 14 (+/- 3 days)	Day 21 (+/- 3 days)	Day 28 (+/- 4 days)		
Written Informed Consent	X						
Subject Demography	X						
Medical History	X						
Disease History	X						
Therapy History	X						
Concomitant Medication(s)	X						X
Physical Examination	X						X
ECOG status, Vital Signs	X						X
Adverse Events	X	X	X	X			X
Chemistry, CBC with Differential, and Vit 25(OH)D3	X				X		X
Pregnancy Test	X						
Recut Tissue/Tissue Block submission confirmation		X				X	
Proliferation index(Ki-67, PCNA), IHC score, Markers of autophagy (LC3B, ATG7), Calcium efflux marker (PMCA2)		X				X	
Assess Invest. Product compliance			X	X			X
Plan of care after surgery							X

Study CRF completion guidelines:

1. Enrollment. If subject is determined to qualify for the study based on their pre-study evaluation.
2. Baseline medical history: a complete medical history will be taken at screening and updated at baseline. Updated medical history will serve as baseline medical history.
3. Baseline symptoms review: a complete system review and symptoms assessment
4. Physical exam: a complete physical examination will be performed at visit specified in Table 4.
5. Vital signs: vital signs will be performed per visit schedule; vital signs might include height, weight, body temperature, respiratory rate, blood pressure, pulse rate and will be assessed per practice standard procedures.
6. Laboratory tests: include chemistry, CBC with differential, and vitamin D levels, performed at screening and at the end of treatment.
7. Pregnancy test: will be performed for women of childbearing potential at baseline and post treatment as part of standard of care.

8. Adverse events: all adverse events will be recorded in CRFs and assessed by investigator or qualified personnel.

Data must be entered within 30 days of patient's study visit

11.0 Pharmaceutical and Investigational Device Information

Vitamin D3 will be provided to the patients by the sponsor of the study.

Patients will take 100,000IU loading dose of vitamin D3 at Day 1. The loading dose must be taken by the patient while at the investigator's site. The loading dose will be dispensed as 10 capsules of 10,000IU/capsule.

Patients will be dispensed 32 capsules / 1 bottle (4,000IU/1 capsule) of vitamin D3 at Day 1 visit to take home with them. Bottle must be labeled with instructions on how to take the drug as well as the assigned patient ID number. They will be instructed to take 1 capsule per day, with water, for 30 days, using the dispensed study bottle. Patients will be instructed to stop taking their daily vitamin D3 dose after 28 days (+/- 4 days) of treatment. A study drug diary will be provided for all patients participating in the study.

12.0 Statistical Considerations

This pilot study will explore the biologic impact of vitamin D supplementation in female patients with DCIS and LCIS.

12.1 ENDPOINTS

PRIMARY ENDPOINT
The primary endpoint of the study is Ki 67 measured from patients' tissue biopsy.

SECONDARY ENDPOINTS
The secondary endpoints are the levels of proteins of the autophagy pathway (LC3B, ATG7), levels of the Calcium transport proteins (PMCA2), and HER2 localization.

12.2 Study Design

All eligible patients entering the study will receive a 28 days (+/- 4 days) schedule of vitamin D supplementation and then proceed with the standard of care of surgical excision. Immunohistochemistry studies of the same patient's initial tissue from the patient's diagnostic biopsy (baseline), and from the breast surgical specimen (post-treatment) will be compared.

12.3 Sample size

This is a pilot study with a proposed sample size of 15 patients. Previous research by Chen et al 2009, suggests that a mean reduction of Ki67 between neoadjuvant therapy and the baseline is about 10.2%. Based on their published data, we have powered our study for a 10% mean reduction of our primary endpoint Ki67. A sample size of 15 would provide >90% power to test a 10% mean change with a standard deviation (SD) of 1.8% for the difference between the baseline and after the treatment, assuming a one-sided test with an alpha level of 0.05. With a larger SD of 12% of the mean change, the sample size of 15 would still provide 80% to detect the 10% mean change.

12.4 Analysis plan

Descriptive statistics (N, mean, median, Min, Max, STD for continuous variables, and N, proportion for categorical variables) will be used to summarize patients' demographics as well as lab results. The proliferation index measured by Ki67 will be described for both baseline (pre) and surgical (post) vitamin D supplementation. A paired t-test or the non-parametric Wilcoxon signed-rank test will be used when appropriate to compare patients' outcome between baseline and after-treatment. A p-value of <0.05 will be considered statistically significant. Since this is a pilot study, no adjustment for the multiple comparisons will be made. SAS software Version 9.3 (SAS Ins, Cary NC) will be used for statistical analysis.

Safety and Tolerability

The assessment of safety will be based on the frequency of adverse events and on the number of significant laboratory abnormalities. Adverse events will be summarized as the number and percentage of patients having any adverse event by body system, type of adverse event, and maximum severity according to the CTC grade (Sec. 8.0). Those adverse events which result in death, discontinuation or are otherwise classified as dose limiting (Sec 8.0) will be presented separately. Tolerability will be assessed using ECOG Performance Status and/or Karnofsky.

13 Ethical and Regulatory Considerations Form

13.2 FDA 1572

Prior to initiating this study, the principal investigators at each site will each provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators that will participate in the protocol.

13.3 Other Required Documents

- Signed and dated current (within two years) CV or biosketch for all investigators listed on the Form FDA 1572 for participating institutions.
- Current medical licenses for all investigators listed on Form FDA 1572 for the participating institutions.
- Lab certification (i.e. CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572
- Documentation of training in "Protection of Human Research Subjects" for all investigators listed on the FDA Form 1572 for the participating institutions.
- Documentation of Federal-wide Assurance number for the participating institutions.
- Facility financial disclosure form from all investigators.

13.4 IRB Approval

Prior to initiating the study and receiving agent, the principal investigator will obtain written approval to conduct the study from the trail site institution IRB. Should changes to the study become necessary, protocol amendments will be submitted to the IRB according to the IRB Amendment Guidelines. The amended protocol must be approved by the IRB prior to implementation. Only one version of the protocol will be the correct version.

Amendments must be initiated through the clinical sites and submitted to the Inova IRB, and the George Mason University IRB for approval.

13.5 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent and potentially other IRB approved materials to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study to each subject. Each subject will be informed that participation in the study is voluntary and that she may withdraw from the study at any time. If the participant decides to participate in the study, 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.

This informed consent will be given by means of a standard written statement, written in non-technical language. The subject will read and consider the statement before signing and dating the consent, and will be given a copy of the signed document. If the subject cannot read or sign the document, an oral presentation will be made. On the consent form, the method through which consent was presented will be documented along with the specific means by which the subject communicated agreement to be a part of the study. An impartial third party will witness the entire consent process and sign the consent document. No patient will enter the study before the informed consent has been obtained.

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. Prior to study initiation, the informed consent document must be reviewed and approved by the investigators and the IRB at each institution at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by the Inova IRB and then each institution prior to initiation.

13.6 Coordinating Center

The CRC will contact George Mason University for regular tissue transfer and sharing of clinical data. All participating sites will obtain local Institutional Review Board (IRB) approval and provide the Principal Investigator with copies of the initial local IRB approval, protocol amendment approvals and annual continuing review approvals. The study coordinator will send electronic reminders to site contact personnel at least 90 days prior to the Continuing Review Anniversary.

13.7 Statement of Anticipated Impact and Benefit

Overall, this study will be conducted in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Furthermore, this study supports the strategic goal to increase translational research and external collaborations with George Mason University. More specifically, it uses the data to specifically answer clinically important questions to cancer development and treatment.

14 References

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15.0 APPENDICES

Appendix 1
Subject Information Sheet & Study Calendar

1. Swallow the study drug (vitamin D3) whole. Do not chew them prior to swallowing.
2. Take 1 capsule per day (4,000IU/capsule), with water, at approximately the same time each day.
3. Vitamin D3 is best absorbed when taken with food containing fat such as a small piece of toast with butter. Don't take it on an empty stomach or in between meal times.
4. Please record the vitamin D3 medication you take each day on your calendar with a check mark. Please note any days you miss medications.
5. If you miss a dose, and discover it on the same day, please take it (e.g. you usually take your pills in the morning, but remember at lunch that you didn't). Do not take two doses at once to catch up.
6. If you vomit after taking a dose you should NOT "make it up" and should resume treatment the next day.
7. If you inadvertently take 1 extra dose during a day, you should NOT take the next day's dose.
8. Please keep the medication in the bottles provided and do not transfer it to any other container.
9. Please record new symptoms on your calendars: when they started and stopped, and if you took any medication to relieve the symptom.
10. Bring your calendars and capsule bottles to every clinic visit. UNUSED drug and/or empty bottles SHOULD BE RETURNED to the site on the Day 28 (+/- 4) visit.
11. If you run out of medication before your next clinic visit, please call us so that we can get you a refill before you run out.
12. If you have any questions, problems, or if you are not feeling well, please contact:

    _____

STUDY DRUG DAILY DIARY
Protocol: An Exploratory Pilot Study of Vitamin D Supplementation in Women with DCIS and/or LCIS

Study Patient ID number: _____

Day 1, date: _____ Dose Taken at Day 1: _____

Signature/Date of CRC documenting Day 1 dose administration: _____

DAY	DATE	Vitamin D3- NUMBER OF CAPSULES TAKEN) (Daily dose is 1 capsule, please add to the columns below a "1" of 1 pill was taken or a "0" if no pills were taken)	COMMENT (e.g. Please use this section to write down any side effect you are experiencing on a given day, explanation for any extra doses taken or missed doses, etc.)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
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24			
25			
26			
27			
28			
29			
30			
31			
32			
Please stop taking vitamin d3 after 28 days (+/- 4 days) of treatment.			

Patient's Signature: _____

Date: _____

Appendix 2: ECOG Performance Status Scale

Grade	ECOG PERFORMANCE STATUS*
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

*Data Source: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Abbreviations: ECOG = Eastern Cooperative Oncology Group