Protocol title: Safety, feasibility and efficacy of vitamin D supplementation in women with metastatic breast cancer (SAFE-D)
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I. Abstract:

Background: Several clinical trials are underway to investigate if variable forms of vitamin D (D_2 vs. D_3) prescribed at different doses (10,000-50,000 IUs/week) can improve the side-effects associated with treatment for estrogen receptor positive (ER+) breast cancer, specifically aromatase inhibitors (AIs.) Presumably for generalizability and potential safety purposes, these trials predominantly exclude women with metastatic breast cancer (MBC); a rapidly expanding sector of the cancer survivor population who experience significant treatment-related side-effects. Evaluation of the safety of vitamin D_3 supplementation is crucial since supplementation can lead to hypercalcemia and importantly, *in vitro* studies have shown that vitamin D_3 influences the transcription of a gene that increases estrogen production. To assure that vitamin D_3 does not abrogate the clinical effects of anti-estrogen therapies, the effect of vitamin D_3 supplements on estrogen production requires an evaluation that further explores and defines its potential role in symptom management for this population.

Objectives: This pilot study will evaluate the feasibility of vitamin D_3 supplementation (n=25) in women with MBC, providing much needed data on the preliminary safety and efficacy of this treatment in this patient population. This study will determine: 1) if weekly supplementation of high dose vitamin D_3 increases serum levels of 25(OH)D without adverse effects *related* to such therapy (primary aim); 2) the effects of vitamin D_3 supplementation on symptom management (secondary aim); and 3) if vitamin D_3 supplementation is associated with improved biomarkers of inflammation (exploratory aim.) Additionally, we will examine the prevalence of symptoms and quality of life in (n=25) women with advanced breast cancer who have normal serum levels to investigate associations with biomarkers of inflammation.

Methods: . Adult, female patients ($\geq$18 years) with ER+ MBC (Stage IV) of any race/ethnicity will be recruited from within and around LUMC. Women with a serum 25(OH)D <30 mg/dl will be invited to participate in an 8 week "proof of concept" study. Following current clinical practice guidelines, eligible participants will receive 50,000 IUs of vitamin D_3 weekly for 8 weeks. Laboratory values (e.g., serum 25(OH)D, calcium, , serum estradiol), muscle function and inflammatory biomarkers (e.g.,CRP) will be examined pre- and post-supplementation, while symptoms (pain, sleep, fatigue, mood, and overall quality of life) will be assessed at baseline, 4 and 8 weeks post-supplementation. We will assess if increases in serum 25(OH)D are associated with clinically significant changes in serum estradiol, improvements in symptoms and QOL, and decreased inflammation, controlling for sunlight exposure, diet, physical activity and body composition. Women with a serum 25(OH)D ≥ 30 mg/dl who do not require supplementation will be invited to complete the study questionnaires at baseline.

Significance/Impact: Vitamin D_3 may be a cost-effective, adjuvant therapy to combat the side-effects of treatment, to increase medication adherence and to enhance supportive care in this unique population in need of evidence-based strategies. These findings will help to characterize symptom burden in this growing population and to demonstrate the feasibility of this clinical trial. Ultimately, these data will provide novel, preliminary data to justify a larger randomized trial.

II. Background

Breast cancer accounts for more than one-fourth of all new cancer diagnoses among women in the United States, with more than 232,000 new cases estimated for the year 2013.[1] Despite the advances in the management of this disease, a significant number of women will go on to develop metastatic breast cancer (MBC), typically reflecting spread to the bone, lungs or viscera. Johnson et al recently showed that the incidence of MBC *at diagnosis* is increasing, showing greater disparities among younger women (aged 25-39) and minority populations.[2] However, the introduction of novel targeted therapies, in particular treatments for HER-2 positive disease, has altered the natural history of advanced breast cancer, with an unprecedented impact on survival from such treatments. As such, the number of women living with MBC will continue to grow, reflecting a unique, rapidly expanding sector of the cancer survivor population with considerable supportive care needs that are largely unknown.

a. MBC and QOL: Cancer-related QOL is a complex entity, taking into account physical, psychological, social and sexual factors.[3] It is an important outcome measure in all patients with breast cancer, but it is critical in the metastatic population since the disease is no longer considered curative and treatments are dictated by QOL. Although QOL has been examined extensively in women with early stage breast cancer, fewer studies have focused on women with MBC. Using a cross-sectional study design, Reed et al examined the QOL and supportive needs of women (n=235) with MBC. QOL was significantly lower in women with MBC compared to women with earlier stage disease (89.0 ± 21.8 vs. 112.8 ± 20.9, respectively). Thirty-four percent reported high levels of pain and 38% complained of significant fatigue, suggesting that symptom control was inadequate.[4] In a smaller study, Meisel et al reported good QOL among long-term (>5 years) survivors of MBC (n=18), yet these women reported significant anxiety and depression. Additional work is needed to more comprehensively identify symptoms and to implement and test appropriately tailored therapies.

b. MBC and vitamin D: Low levels of serum 25(OH)D at the time of diagnosis have been shown to predict recurrence and death,[5,6] underscoring the clinical significance of widespread vitamin D deficiency/insufficiency reported among breast cancer survivors.[7-9] Purportedly 1,25 $(OH)_2D$ has been show to induce cell-cycle arrest, increase expression of receptor activator of nuclear factor κB ligand, and down regulate the expression of cyclooxygenase-2.[10] Hence, sufficient levels of vitamin D are needed to favorably impact breast cancer biology. In women previously diagnosed and treated for breast cancer, the prevalence of vitamin D deficiency/insufficiency is ~75%, yet these studies predominantly focused on women with early disease (Stage I-III).[8,9] Vitamin D studies conducted in women with MBC are limited, but report similar prevalence estimates with lower overall levels of serum vitamin D.[11,12]

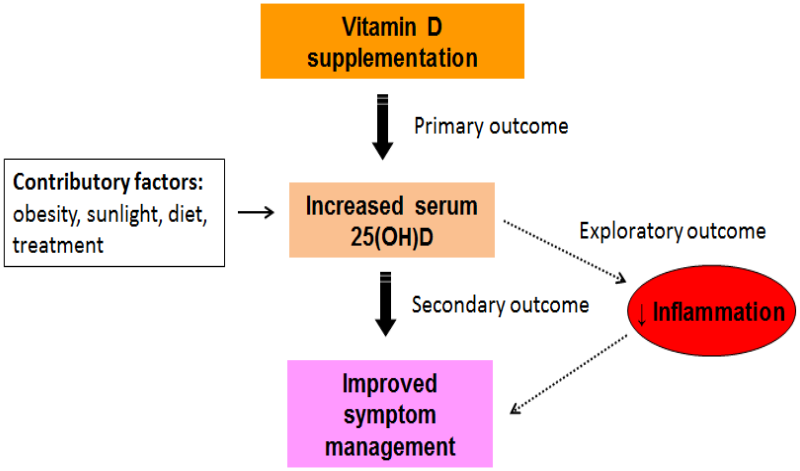
c. Breast cancer treatment and symptom management: For women with estrogen receptor positive (ER+) disease, aromatase inhibitors are considered a frontline therapy, since they efficiently and effectively decrease circulating estrogen by halting the conversion of androgens to estrogen. Unfortunately, arthlagias and myalgias are highly prevalent within weeks of initiation and may result in discontinuation.[13] The etiology of these musculoskeletal disturbances is not well understood; however, it has been postulated that estrogen itself has analgesic properties[14] and that cytokines, such as Il-1, TNF-α, are suppressed with higher levels of estrogen.[15] It has been further suggested that cartilage turnover increases as estrogen decreases,[16] and that the combination of efficient estrogen deprivation combined with low serum 25(OH)D accentuates muscle and joint pain.[7] Thus, low levels of estrogen are associated with reduced pain tolerance and increased inflammation. Additionally, breast cancer treatment is accompanied by a myriad of endocrine symptoms, including weight gain, fatigue and mood swings. In non-breast cancer populations, vitamin D supplementation has been shown to regulate weight,[17] mood[18] and to decrease inflammation.[19]

d. **Significance:** A diagnosis of MBC is accompanied by a multitude of physiologic and psychological sequela.[20] Managing symptoms to maintain an optimum quality of life (QOL) is the major goal of care in the metastatic setting because all therapy is considered palliative. For women with ER+ breast cancer

(~75% of cases), therapies primarily target decreasing the production and absorption of estrogen. These reductions in circulating estrogen are associated with significant bone loss, arthralgia and myalgia,[21-23] limiting adherence and compromising QOL. The pathophysiology of these side-effects may involve sub-optimal vitamin D status, which may be more pronounced in a hypoestrogenic environment.[7] Therefore, supplementation with vitamin D_3 *(*cholecalciferol) shows promise as a cost-effective, adjuvant therapy to combat the side-effects of treatment, to increase medication adherence and to enhance supportive care. However, preliminary data are critical to demonstrating the safety of high dose vitamin D_3 supplementation in this unique patient population and to evaluate if this therapy has a favorable impact on symptom management and possibly inflammation. Ultimately, this study could provide insights into improving QOL and offer novel, preliminary data to justify a larger randomized trial.

e. Conceptualization

For this study, we hypothesize that low levels of vitamin D are highly prevalent among women with MBC and that repletion to achieve serum 25(OH)D levels ≥ 30 mg/dl will be safe. We are also postulating that the therapeutic effects of serum vitamin D normalization will be associated with improvements in symptom management and biomarkers of inflammation. (Depicted in Figure 1) The association between vitamin D deficiency and inflammation has been recognized from observational studies[24] and administration of vitamin D has been shown to improve inflammatory markers in non-cancer patient populations.[19,25] Mechanistically, 1,25-dihydroxyvitamin D_3 (the active form of vitamin D) inhibits the production of pro-inflammatory cytokines (ex, IL-6, IL-1β, TNFα) leading to reduced T cell activation and reduced secretion of these cytokines.[26]



f. Preliminary work:

Prior work in women with MBC: To explore the diet and physical activity habits of women with MBC, we recruited 25 women with advanced disease from two university hospitals over a 4 month period. Eligible women were identified by their medical oncologist (Drs. Kent Hoskins and Ruta Rao) and referred to Dr. Sheean (PI). Demographic and medical information was collected, and questionnaires were completed to quantify nutritional symptoms, physical activity, quality of life, functional performance and dietary intake. Participants were 58.8 (±12.7) years of age, predominantly minority (n=15) and had been living with MBC for 37 (± 29) months. Dietary supplements were used by 68% (n=17) of women. The majority had an Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (n=20) and most (n=13) had only received one or two different drug therapies to treat their disease, signifying a relatively healthy subset of MBC patients.

Overall QOL was lower when compared to other women with breast cancer (107 ± 20 vs. 119 ± 18) but higher compared to other women with MBC (107 ± 20 vs. 89 ± 22),[4] respectively. These participants displayed lower physical well-being compared to other breast cancer survivors (22 ± 5 vs. 24 ± 5, p=0.05), with 92% (n=23) of women reporting pain symptoms and 60% (n=15) reporting fatigue. We acknowledge that patients with greater disease acuity were not approached for study inclusion, reflecting an inherent selection bias. As such, we believe our findings overestimate QOL and underestimate symptom reports in the broader MBC population. Despite these limitations, we believe these findings highlight that interventions targeting the supportive care needs of these women are greatly needed and desired.

III. Study Aims

Several clinical trials are underway to investigate if vitamin D_2 or D_3 provided at various doses (10,000-50,000 IUs/week) can improve the side-effects associated with anti-estrogen therapies, specifically aromatase inhibitors (AIs). However, these current trials use variable forms of vitamin D and predominantly include women with Stage I-III disease, excluding women with metastatic breast cancer. Evaluation of the safety of vitamin D_3 supplementation is crucial since supplementation can lead to hypercalcemia and importantly, *in vitro* studies have shown that vitamin D_3 influences the transcription of a gene that increases estrogen production.[27,28] To assure that vitamin D_3 does not abrogate the clinical effects of anti-estrogen therapies, the effect of vitamin D_3 supplementation on estrogen production requires evaluation. Therefore, the overarching goal of this pilot study is to evaluate the safety, feasibility and efficacy of vitamin D_3 supplementation in women with MBC. We will address and test the following aims and hypotheses, respectively:

Aim 1: To determine if weekly supplementation of 50,000 IUs of vitamin D_3 raises serum levels of 25(OH)D to >30 mg/dl without adverse effects.

Hypothesis 1: Women who are compliant with vitamin D_3 supplementation, as evidenced by normalization (>30 mg/dl) or increases in their serum 25(OH)D levels, will not experience significant changes in serum calcium or serum estradiol levels.

Aim 2: To determine the effect of vitamin D_3 supplementation on symptom management.

Hypothesis 2: Women who achieve serum concentrations of 25 (OH)D ≥30 mg/dl or experience significant increases in 25(OH)D will exhibit improvements in pain, fatigue, sleep, mood, muscle function and overall quality of life.

Exploratory Aim: To explore the mechanistic effects of serum vitamin D levels and vitamin D_3 supplementation on inflammatory markers and potential associations with symptom management.

Summary: Evidence from studies involving early stage breast cancer participants confirms that musculoskeletal pain, endocrine related symptoms and mood disturbances are commonly associated with breast cancer treatment, particularly hormone deprivation therapies. The high prevalence of vitamin D deficiency/insufficiency among breast cancer survivors is well accepted and further hypothesized to aggravate treatment-related side effects, particularly arthralgias. Women with MBC are excluded from the majority of on-going vitamin D supplementation trials for safety and generalizability purposes. However, novel therapies are continuing to improve and prolong the lives of these women, resulting in a rapidly expansive group of breast cancer survivors. While vitamin D supplementation is prescribed to correct an underlying nutrient deficiency in the clinical context of preserving bone health, emerging evidence suggests it may have more systemic effects. Thus, vitamin D repletion/supplementation has profound potential implications for women with MBC, whose primary goal of treatment is to minimize the side-effects of treatment in support of optimal quality of life. This study reflects a highly innovative, yet simple therapy that could ultimately provide these survivors with a much needed evidence-based supportive care strategies.

IV. Administrative Organization

This study involves collaboration among investigators and support staff from the School of Nursing, the Department of Medicine, Hematology/Oncology and Public Health Sciences divisions, the Cardinal Bernadin Cancer Center, the Loyola University Medical Center (LUMC) Pharmacy and the Clinical Research Office (CRO). This investigation will be conducted on the Loyola University Health Sciences Campus. Participants will be evaluated in the Bernadin Cancer Center and in a devoted research space within the Maguire Building utilizing the nursing expertise offered by the School of Nursing research staff.

Laboratory analyses will be completed in the Core Laboratory of LUHS, within a Basic Science Laboratory at LUHS and/or outsourced to Quest Diagnostics Biospecimens will be stored in the biorepository supported by the CRO (LU# 204853). Additionally, the CRO will offer biostatistical support, data management oversight and regulatory services required for this trial. The LUMC Pharmacy will provide and dispense the study medication and the Public Health Science division will provide the machinery and technical expertise for body composition assessment. Because participants may be referred from surrounding oncologists, this study will leverage existing relationships among investigators to promote collaborations across institutions. The investigators on this study reflect a multidisciplinary team with expertise in medicine, nutrition, nursing, oncology, biostatistics, and public health.

V. Study Design

a. Research design: This study is comprised of an intervention arm (Group 1) and non-intervention arm (Group 2). For eligible women with low serum levels of vitamin D, we propose an 8 week open label, single arm "proof of concept" study to address concerns regarding vitamin D_3 dosing, to monitor important laboratory parameters and to assess potential effects on short-term outcomes. While a randomized controlled trial (RCT) has been the traditional model to evaluate the efficacy of vitamin D supplementation, this design may be considered premature until the safety of this therapy has been evaluated in a patient population with the potential for high clinical acuity. For the women who meet all eligibility criteria except they have normal serum levels of vitamin D (Group 2), we will assess their symptomology and test associations with the biomarkers of inflammation.

b. Study population: Adult females with ER+ MBC can participate in the present study provided they are under the care of medical oncologist and meet all eligibility criteria. Women with early stage disease (Stages 0-3) or women with MBC who are ER- are unable to participate in this study.

c. Sample size and power considerations: Because this is a pilot project that will assess the feasibility, preliminary safety and short-term efficacy of a vitamin D_3 interventionin this population, typical sample size calculations will not be employed. We will assess symptoms at baseline for all participants to establish prevalence estimates and at three time points in the intervention group to help increase our abilities to detect changes over time. Because of the high prevalence of vitamin D deficiency among breast cancer survivors (~75%) and the high volume of patients seen at LUMCand by Dr. Rao (collaborator), we anticipate that we can successfully recruit and enroll 50 women who meet the eligibility criteria. Our goal is to recruit 25 women for the intervention arm. Factoring in an attrition rate of ~20%, we should have complete data on 20 women who complete the intervention. This sample size is sufficient to demonstrate feasibility and will allow for the generation of effect size and variance estimates, as well as confidence interval widths around these estimates for future applications.

d. Study endpoints: The primary endpoint in this study is serum 25(OH)D. We will use this serum biomarker to assess if repletion levels are obtained (≥30 ng/dl), to test associations with symptoms and/or to assess the degree of change from baseline to follow up. Serum calcium and serum estradiol levels will be monitored to be sure these levels normalize or to assess clinically significant changes following supplementation, respectively.

To address our secondary endpoints, we will be assessing symptoms, including pain (Brief Pain Inventory; Cleeland,1994; FACT-Bone pain, Broom, 2009), fatigue (Piper Fatigue Scale; Piper, 1998), mood (Hospital Anxiety and Depression Scale; Herrmann, 1997; Patient Health Questionnaire-8; Kroenke, 2001), muscle function (handgrip strength), sleep (Pittsburg Quality Sleep Index; Carpenter, 1998), overall health and QOL (FACT-B; Brady, 1997; FACT-ES; Fallowfield, 1999.) These specific tools were selected because they have been utilized in breast cancer populations and/or other cancer populations and will provide the ability to compare our findings to others. In addition, to address important confounders, we will gather data on diet (Block Calcium/Vitamin D screener; Cummings, 1987), physical activity (Godin Leisure Time Activity, 1985), body composition (bioelectrical impedance, dual energy x-ray

absorptiometry), sunlight exposure (Glanz sunlight exposure, 2008), social support (Multiple Outcomes Social Support; Sherbourne, 1991) and previous/current breast cancer treatments.

VI. Study Procedures

a. Study subject procedures: Subjects must meet all of the inclusion and none of the exclusion criteria to be enrolled into the intervention arm of the study. In addition, women meeting all of the inclusion and exclusion criteria except the serum 25(OH) cut point will be offered the opportunity to participate in the cross-sectional arm of the study.

i. The following criteria will be used to assess eligibility and participation.

Inclusion Criteria:

1. Metastatic breast cancer (Stage IV)
2. Histologically confirmed estrogen receptor positive disease
3. Female
4. Serum 25(OH) <30 ng/ml
5. Age ≥ 18 years
6. Pre or post-menopausal
7. ECOG Performance status 0-2
8. Adequate organ function as defined as GFR> 30 mls/min and serum calcium ≤ 10.4 mg/dl
9. Any race/ethnicity
10. English speaking
11. No changes to MBC treatments within 30 days of enrollment and/or deemed clinically stable by their treating physician
12. Willingness to sign a written informed consent and complete questionnaires
13. Cease ingestion of vitamin D supplementation not study related

Exclusion Criteria:

1. Women with Stage I-III breast cancer
2. Serum 25(OH)D levels ≥ 30 ng/ml
3. Untreated CNS involvement
4. History of kidney stones
5. History of renal failure
6. History of hyperparathyroidism
7. History of hypersensitivity to vitamin D
8. Non-English speaking
9. Currently pregnant or lactating, or anticipating pregnancy
10. Unwilling to cease ingestion of calcium supplements (>1000 mg/d)
11. Unwilling or unable to complete informed consent or study questionnaires
12. Psychiatric or other clinical conditions that preclude study compliance
13. Other important medical or safety considerations at the discretion of the investigator and/or study physician, including non-compliance with the study therapy or other activities

ii. Recruitment sites and recruitment: Women with MBC will be recruited from Cardinal Bernadin Cancer Center at LUMC with the direct assistance of the research nurses in the Breast Oncology Clinic

and support from the medical oncologists who treat these women. Participants will be informed of the study verbally and provided with a study brochure during their clinic appointment. Interested participants will be contacted by the study staff to explain the study in more detail and answer questions. In order to inform a broader sample of patients from LUMC about the study, we will send letters describing the investigation to the appropriate patients under the care of LUMC medical oncologists. An IRB approved waiver of HIPPA authorization will be used to identify patients from these physicians who meet the study criteria using CPT codes. We will provide flyers in the breast oncology clinic and surrounding clinics for potential participants to contact us. In addition, Dr. Ruta Rao, a medical oncologist at Rush University Medical Center (RUMC), will verbally inform her patients about the study, provide handouts to those who express interest, and refer them to Dr. Sheean for further evaluation. (Drs. Rao and Sheean successfully collaborated on a previous pilot described in Preliminary work.) Women with confirmed interest will be screened via telephone to see if they can be scheduled to return to the research office for consent and baseline study assessment.

iii. Initial screening: Referred participants will be phoned or seen in clinic to describe the study. If interested, age, health information and questions regarding vitamin D supplementation will be asked. If any of the exclusion criteria are reported, they will not be able to continue their participation. If initially eligible, they will be scheduled for a blood draw.

iv. Baseline screening and enrollment: Interested participants will be consented by the PI or study nurse in a private room, assigned a unique study identification number and undergo phlebotomy. Participants will have their blood drawn in the Cardinal Bernadin Cancer Center Phlebotomy Laboratory to determine serum levels of vitamin D and other relevant laboratory values (Refer to Measurements.) Participants will in the non-intervention arm (Group 2) will be asked to complete the questionnaires, either in person, over the phone or via mail.) The research study staff with review the results of the serum vitamin D blood tests and confer with the medical oncologist regarding further participation in the study. Participants who are vitamin D deficient (<30 mg/dl) will return to LUMC to participate in the intervention arm (Group 1), to complete questionnaires and to receive the study medication.

Optional Biobanking. At the study completion visit, participants may *optionally* agree to reposit up to two tablespoons of blood into CRO-BIOREP (LU# 204853) for future research purposes. Participants will sign a second informed consent document should they agree to reposit their blood. The research team members and Biorepository staff will each retain a copy of the signed repository informed consent document. It is important to point out that participants do not need to agree to reposit blood in order to participate in the trial.

All patients who agree to reposit blood specimens into CRO-BIOREP (LU# 204853) will be assigned a unique code using our SMART-ID system. This number will be used to connect coded specimens and coded data to other studies conducted by the Clinical Research Office in which they may participate. To receive this number, we will ask participants for sex at birth, month of birth, day of birth, year of birth, and social security number. This information is entered into a secure website where it is hashed into a unique ID using a java script. The participant and research team will receive the unique ID number. Once the number is created, the information that was entered is immediately deleted. This means the website will not retain any of the information that is entered to create the SMART-ID. However, should anyone need to retrieve a SMART-ID number, you will be able to get it again at a later date by going to the website and entering the same information (i.e., sex, date of birth, and social security number) that was entered before.

Freezerworks, the biospecimen tracking software used in the repository, will also automatically assign specimen numbers to incoming specimens. These numbers will identify all donated specimens. The list

that links these codes with patients' identifying information will be kept separate from all research specimens and clinical data.

Information that may readily identify patients will never be shared with anyone.

v. Study intervention: Following the clinical practice guidelines of the Endocrine Society, participants in Group 1 will receive 50,000 IU of vitamin D_3 once a week for 8 weeks.[29] This dose of vitamin D_3 is similar to those used in other breast cancer trials involving women with Stage 0-III disease, and should be sufficient to increase levels in normal weight, overweight and obese women within an 8 week time frame. In addition, weekly dosing will also help to increase study compliance and participants will receive a weekly reminder (phone call, email or text) to take their supplement. Because this is a single arm, open label trial, all participants will be aware that they are taking the study medication and that no placebo is being offered in this trial. Participants will be encouraged to take less than 1000 mg/d of calcium supplementation and to emphasize food sources of calcium (see calcium handout in the appendices). Participants in the non-intervention arm (Group 2) will complete all of the baseline (Week 0) study questionnaires and physical measures.

vi. Study assessments and activities: Guided by the Wilson and Clearly health-related QOL model,[30] we will examine seven levels of patient outcomes, including: a) biological and physiological, b) symptom status, c) functional status, d) health perceptions, e) overall QOL, and f) characteristics of the individual and the environment. Variables, measures and times are delineated below (Table 1). Brief details about each of the measurement tools are described below.

Table 1. SAFE-D study variable, measures, constructs and timing

	Variables	**Measures**	**Wilson & Cleary construct**	**Week**
Aim 1	**Low vitamin D (Primary)**	25(OH)D, calcium, , serum estradiol, CMP	Biological	0,8
Aim 2	**General well-being (Secondary)**			
	Pain	Brief Pain Inventory, FACT-Bone pain	Symptom status	0,4,8
	Fatigue	Piper Fatigue Scale	Symptom status	0,4,8
	Mood	HADS, PHQ8	Symptom status	0,4,8
	Muscle function	Hand grip strength	Functional status	0,8
	Performance status	ECOG	Functional status	0
	Sleep	PSQI	Symptom status	0,4,8
	Overall health	Single item question	Health perceptions	0,4,8
	QOL	FACT-B, FACT-ES	Overall QOL	0,8
	Serum vitamin d and Inflammation (Exploratory)	CRP, interleukins, TNF-α	Biological	0,8
Other Factors	Demographics and cancer history	Age, race, employment, home life, treatments	Individual, Physiological	0
	Diet	Calcium/Vit D & Fruit, vegetable, fiber screeners	Biological	0
	Obesity	Body composition, Anthropometrics	Physiological	0,8
	Physical activity	Godin	Physiological	0,8
	Support	MOS Social Support	Environmental	0,8
	Sunlight	Sun exposure tool	Physiological	0,8

vii. Laboratory Outcomes: Laboratory analyses will be conducted by the Core Laboratory of LUHS, within a Basic Science Laboratory at LUHS and/or by Quest Diagnositics. Serum vitamin D and serum

estradiol will be measured using liquid chromatography/tandem mass spectrometry (LC-MS/MS). This technique is considered the 'gold standard,' providing total 25(OH)D which includes vitamin D_2 and vitamin D_3, as well as total serum estradiol levels Calcium will be quantified by spectrophotometry Biomarkers of inflammation (e.g., C-reactive protein (high sensitivity), interleukins, TNF-α) will be measured at baseline and follow up with the laboratory analyses conducted in batch to decrease intra-assay variability. The comprehensive metabolic panel (CMP) will be completed to assess baseline organ function, and includes albumin, alkaline phosphatase, ALT, AST, calcium, CO_2, BUN/Creatinine, chloride, creatinine with estimated GFR, globulin (calculated), glucose, urea nitrogen, potassium, sodium, total bilirubin, and total protein.

viii. General well-being outcomes: To increase comparability across studies, the selected tools have been used previously in breast cancer and/or other cancer populations. (Copies of the tools can be found in the Appendices.)

Demographics and treatment: Similar to our previous study, we will obtain information on age, race/ethnicity, employment status, living situation, diagnosis, treatments (medical, surgical and radiation) and metastatic sites.

Brief Pain Inventory-Short Form: This validated instrument was designed to assess pain in cancer patients.[31] It includes four items measuring the severity of pain in the last 24 hours on a scale of 0–10, plus seven additional items measuring the extent to which pain interferes with life activities.

Functional Assessment of Cancer Therapy-Bone Pain (FACT-BP): This 16-item questionnaire was developed to assess bone pain. It was validated in women with metastatic breast cancer involving the bone.[32] It is scored similarly to other FACT instruments (range 0-4), takes ~5 minutes to complete and has been used in a previous vitamin D supplementation trial for cross-comparison.[11]

Piper Fatigue Scale (PFS): The PFS is composed of 22 numerically scaled, "0" to "10" items that measure four dimensions of subjective fatigue: behavioral/severity (6 items); affective meaning (5 items); sensory (5 items); and cognitive/mood (6 items.) Four sub-scale/dimensional scores and total fatigue scores can be calculated to provide reliable, valid assessments of fatigue in breast cancer patients.[33]

Hospital Anxiety and Depression Scale (HADS): The HADS is a validated, fourteen item scale with 7 items to categorize anxiety and 7 items to measure depression.[34] Each item on the questionnaire is scored from 0-3, meaning a person can score between 0 and 21 for either anxiety or depression.

Patient Health Questionnaire 8 (PHQ8): The PHQ8 is a reliable and valid measure of depression severity.[35] It has 8 questions, each scored 0-3. The sum of all 8 items is used to determine depression, with scores of 10-20 considered major depression and >20 is severe major depression.

Handgrip strength: Handgrip strength is measured with a handgrip dynamometer (Jamar) which tests grip strength up to 90 kg. It is often used as a test of skeletal muscle function and correlates with total body muscle strength[36] and mortality.[37]

Eastern Cooperative Oncology Group Performance Status (ECOG): This scale is used by clinicians and researchers to assess globally assess disease progress, the impact of the disease on daily living abilities and helps guide appropriate treatment and prognosis.[38] Grades range from 0-5 with scores of 0-2 reflecting full activity to compromises in self-care.

Pittsburg Sleep Quality Index (PSQI): This tool contains 19 self-rated questions (7 component scores, ranging from 0-3) and has been validated in a subset of breast cancer patients (Cronbach's alpha 0.8).[39]

In all cases a score of “0” indicates no difficulty, while a score of “3” indicates severe difficulty. The 7 component scores are added to yield a “global” score (range 0-21).

Overall health: Using a single item, participants will be asked, “How would you rate your overall health at this time?” This global question format is based on national survey data and scored 1-5 (1=excellent, 2=very good, 3= good, 4=fair and 5=poor.)

Functional Assessment of Cancer Therapy-Breast and Endocrine Symptoms: The FACT-B and FACT-ES are widely-used tools that measures multidimensional QOL in patients with breast cancer. They have good reliability, validity and are sensitive to change.[3] There are four QOL subscales: Physical Well-Being, Social/Family Well-Being, Emotional Well-Being, and Functional Well-Being with additional breast cancer and endocrine subscales. Higher scores indicate better QOL and total and subscale scores can be compared to normative scores in breast cancer patients with early and advanced disease.[4,40]

Body composition: This will be assessed using three methods. First, weight, height, waist and hip circumference measures will be taken to calculate body mass index and estimate android and gluteal adiposity. Bioelectrical impedance will be used to assess adiposity at baseline for all participants due to its ease and low costs. Finally, participants will undergo dual energy x-ray absorptiometry (DXA) imaging using the Discovery W by Hologic, located down the hall from the study exam room. This noninvasive, imaging technique provides precise whole-body measurements of fat (total and visceral) and lean tissue, only takes 10 minutes to complete and will be completed on Group 1 participants at their final study visit.

Dietary intake: Because dietary sources of vitamin D are restricted to a limited number of foods, precise intakes are easily obtained and quantified with a short measurement tool. The Block Calcium/Vitamin D screener is a brief dietary assessment tool validated and developed from the National Health and Nutrition Examination Survey 1999-2001 dietary recall data.[41] This screener takes 5-7 minutes to complete and includes 19 food items, 3 supplement questions, and adjusts for food fortification practices. To help further characterize dietary intake, participants will also complete the fruit, vegetable and fiber screener. This screener contains 7 questions and takes 3-5 minutes to complete.

Sun exposure: Because sunlight constitutes the major source of vitamin D, the adult sun habits questionnaire by Glanz et al will be used.[42] Two questions inquiring about summer sun exposure will be used quantify time spent in the sun, which directly correlates with serum vitamin D levels.

Godin Exercise Leisure-time Questionnaire: This 4-item tool allows for the quick quantification of time spent engaged in strenuous, moderate or light activities over a typical 7-day period showing reliability coefficients of 0.8 with $V0_2$ measures.[43] A weekly leisure activity score is generated, as well as the frequency of leisurely activities. This tool is recommended and made available by the NCI.

Medical Outcomes Study Social Support Survey: The self-administered survey is a 19-item multi-dimensional scale assessing four aspects of social support: tangible support, affectionate, positive social interaction and emotional/informational support (Cronbach’s alphas >0.9 for all subscales.)[44] Total and subscale scores are obtained and compared, with higher scores indicating more support.

ix. Study completion: Participants in the intervention will return to LUMC after 8 weeks of supplementation for their final blood analyses (serum 25(OH)D, calcium, , serum estradiol, inflammatory markers), anthropometric and body composition evaluations, pill counts and questionnaire completion. An 8 week intervention was selected because: 1) this timeframe is consistent with the clinical practice guidelines of the Endocrine Society for vitamin D repletion, and 2) it is an efficient and opportune time to assess the effects of vitamin D on biological and symptom outcomes. Participants in the non-intervention arm will not be followed after their baseline visit. They will be considered “complete” after their baseline visit.

x. Patient participation and compensation: We anticipate that we can screen, recruit, and ultimately enroll 50 women over a 12 month time period (n=25 in Group 1, n=25 in Group 2) using patients at LUMC and those referred from RUMC. To enhance retention, participants will be scheduled on a day that is convenient for them and parking tokens will be provided to offset any costs of parking. Honorarium for data collection will be given in a graduated manner [$20 baseline, $30 follow up (Group1 only)]. To enhance medication compliance in the intervention arm, participants will be contacted weekly (phone call, text or email) and reminded to take their vitamin D supplements. For persons who drop out, we will assess the reason for study discontinuation and notify their medical oncologists regarding any recent laboratory findings, as appropriate.

VII. Safety Monitoring Plan

a. Published reports suggest that 50,000 IUs of vitamin D taken ***daily*** can increase vitamin D levels to more than 150 ng/dl and cause hypercalcemia.[45] The proposed dosing for this study is 50,000 IUs per week, which equates to ~7,000 IUs/day. This dose has minimal side-effects in healthy populations, but may include bone pain, constipation, dry mouth, headache, stomach upset and loss of appetite. We will be monitoring laboratory values at baseline and 8 weeks, and patient symptoms at baseline, 4 and 8 weeks.

i. Any patient who receives treatment on this protocol will be evaluable for toxicity. This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 available at (http://ctep.cancer.gov). Participants will be called after 4 weeks of supplementation to ascertain pill counts, gauge their general tolerance and capture symptom management data related to pain, fatigue, mood, sleep and overall health. Although toxicity and adverse side-effects from vitamin D_3 supplement are rare, participants will be queried their general tolerance to the study medication during the 4 week phone call. The following criteria will be utilized to determine supplement suspension or supplement discontinuation: .If the participant develops Grade 1 toxicity that is felt to be possibly, probably, or definitely related to study supplement, the participant will continue to be monitored.

ii. If the participant develops Grade 2 toxicity that is felt to be possibly, probably, or definitely related to study supplement, the participant may go on study supplement holiday until toxicities are Grade 1 or lower (for no longer than 4 consecutive weeks).

iii. If a participant develops any Grade 3 or Grade 4 or has persistent Grade 2 toxicity beyond four consecutive weeks possibly, probably, or definitely related to the study supplement, the study supplement will be discontinued. A blood draw at the nearest Quest Diagnostics facility or the Cardinal Bernadin Cancer Center Phlebotomy Laboratory will be procured to obtain 25(OH)D and a comprehensive metabolic profile.

iv. If a participant's serum calcium and corrected calcium* exceeds 10.5 mg/dL, then study supplement will be suspended. The participant will be encouraged to lower any calcium intake and provided a handout on calcium sources. The serum calcium will be rechecked in two weeks and the participant may continue on study supplement if the serum calcium is below 10.5 mg/dL. Otherwise, the participant will be removed from study intervention. The participant may also be removed from study intervention for any Grade 2 or higher hypercalcemia (serum calcium > 11.5 mg/dl).

*Corrected calcium = serum calcium + [(4.0 – serum albumin) x 0.8]

v. In each of these cases, the follow up questionnaires and follow-up blood analyses will still be collected as per protocol eight weeks after the initial start of the intervention.

b. Participants may be re-challenged following a suspension. Specifically, participants who have had a study physician-ordered dose suspension may be re-challenged by returning to the full dosage, or

the study physician may decide to have the participant continue her participation on a reduced dose (e.g., 25,000 IUs/week) until study completion.

c. A participant will be taken off study if she experiences any of the following adverse events: 1) hypercalemia (>11.5 mg/dL), 2) a serum 25(OH)D level >100 mg/dL with normal calcium or symptoms of vitamin D indicating possible toxicity, 3) clinically significant increases in serum estradiol indicating a potential drug-nutrient interaction, or 4) has intolerable side-effects believed to be associated with vitamin D_3 supplementation. Serious and/or unanticipated adverse events will be reported to the Loyola IRB within 24 hours of occurrence.

d. To ensure the safety of participants, we will complete an exhaustive review of all safety measurements employed in this study after 7, 19, and 25 patients are enrolled. This includes examining their laboratory values, adverse event logs, and notifications in order to make a determination about whether the study should continue for all participants or whether the study should terminate.

VIII. Data Management and Analyses

All hard copies of the data will be kept in a folder, in a locked file cabinet in a locked office in a secure building. Data will be double-entered into appropriate data management software, correcting all data entry errors prior to import into SAS (v 9.2) for statistical analyses.

Standardized descriptive statistics including means, median, standard deviations, frequency and ranges for continuous variables will be calculated to describe the participants and the overall and subscale means for the respective instruments. For specific aim 1, the number of participants who achieve sufficient levels of serum 25(OH)D will be calculated, and paired t tests will be used to assess differences between mean laboratory values from baseline and follow up. Using logistic regression, we will also explore the clinical characteristics of those who achieve sufficient levels vs. those who remain insufficient after the intervention. For specific aim 2, repeated measures analyses will be conducted to examine changes in symptom scales from baseline to interim and follow up.

IX. Other considerations

a. Potential benefits of the proposed research and future directions: Seeking to live as normal a life as possible, including the ability to fulfill roles and maintain relationships, is a goal for many that are negatively affected by MBC. Many patients with MBC have serious concerns, including the fear of dying, declining quality of life, side effects of treatment, the ability to care for family, decreasing physical functioning and care at the end of life. Managing symptoms to maintain an optimum quality of life is the major goal of care in the metastatic setting. Many women with MBC who consume vitamin D take it to 'protect their bones.' This study has the potential to provide preliminary evidence to support that vitamin D levels can improve QOL and enhance survivorship care. Additionally, we will be asking participants to biobank their blood for future studies related to vitamin D. Findings from this study will be used for to design and justify a larger trial.

b. Timeline: This study is designed and intended to be completed within 18 months. We anticipate that we can screen, recruit and enroll 25 women in the intervention in months 1-12. We will enroll women in the cross-sectional arm of this study until the intervention is complete. Data entry will be on-going throughout the study with final data collection will conclude in month 14. In month 15, we will complete data entry, conduct data cleaning and begin initial statistical analyses. In months 15-18, we will complete our statistical analyses and prepare these data for grant, manuscript and oral presentation.

c. This study completed an IND application and has been issued a "study may proceed" decision by the FDA.

X. References

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VII. Appendices

A. Study Instruments

1. Demographics and treatments
2. Brief Pain Inventory- Short form
3. Piper Fatigue Scale
4. Hospital Anxiety and Depression Scale
5. Patient Health Questionnaire 8
6. Eastern Cooperative Oncology Group Performance Status
7. Pittsburg Sleep Quality Index
8. Functional Assessment of Cancer Therapy-Breast and Endocrine Symptoms
9. Sunlight Assessment
10. Block Calcium/Vitamin D screener
11. Godin Exercise Leisure-time Questionnaire
12. Medical Outcomes Study Social Support Survey
13. Food Sources of Calcium
14. FACT-Bone Pain
15. Fruit, vegetable and fiber screener