

Effect of Vitamin D Supplementation on Breast Cancer Biomarkers: CALGB 70806 (Alliance) Study Design and Baseline Data

Ogheneruona Apoe, MD, Sin-Ho Jung, PhD, Heshan Liu, Drew K Seisler, Jayne Charlamb, MD; Patricia Zekan, MD; Lili X. Wang, MD; Gary W. Unzeitig, MD; Judy Garber, MD, James Marshall, PhD, and Marie Wood, MD

Abstract

One in eight women will develop breast cancer over their lifetime with 230,000 women diagnosed in 2015. For this reason, breast cancer prevention efforts are essential. Vitamin D, with anticancer properties, may have a role in prevention of some cancers, including breast cancer. This report discusses the rationale, study protocol, and baseline data for a clinical trial of vitamin D and its effects on breast cancer biomarkers.

This study was a randomized controlled trial designed to evaluate the effect of a fixed dose of vitamin D on specific breast cancer biomarkers. Study participants were randomized to take either vitamin D or placebo for a period of one year. All participants had mammograms and blood drawn for serum biomarkers. A subset of participants underwent random periareolar fine needle aspiration to draw tissue for biomarkers.

From January 2011 to December 2013, 300 premenopausal women, aged 59 or younger, were recruited from 41 institutions across the United States. A total of 102 women underwent random periareolar fine needle aspiration. The last subject completed the trial in January 2015. Baseline vitamin D levels for all participants ranged from 4 to 72 ng/mL, with 62% of participants being vitamin-D deficient at enrollment (≥ 30 ng/mL or ≥ 75 nmol/L). The mean body mass index (BMI) was 27.0 kg/m² (range 15.1–53.6 kg/m²). 14% and 11.7% of participants were Hispanic or African American, respectively.

Accrual and enrollment of participants is feasible for this type of multicenter prevention trial, and it can readily be carried out in a cooperative group setting.

Keywords: biomarkers, breast cancer, breast cancer prevention, chemoprevention, mammographic density, vitamin D

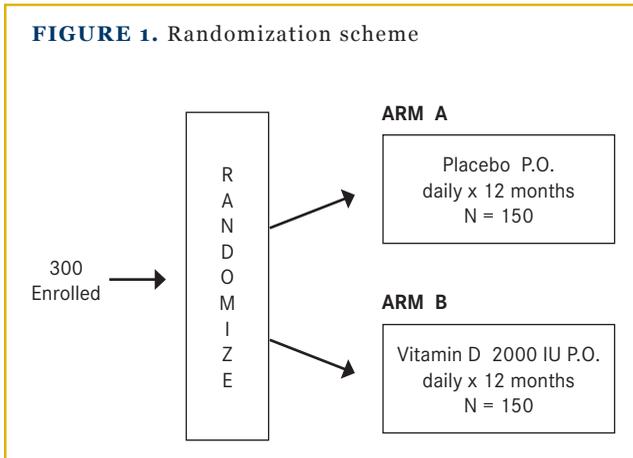
lifetime, with an estimated 231,840 new cases of invasive cancer and 40,290 deaths due to breast cancer in 2015.¹ Early detection through screening mammography has been shown to decrease the mortality of breast cancer by 31%, and the incidence of stage II and higher breast cancers by 25%.² Despite this, breast cancer is still a major cause of morbidity and mortality, and remains the second leading cause of cancer deaths in women. Prevention is key to changing these statistics.

Several agents have been shown to prevent breast cancer. Selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) have been shown to reduce the incidence of breast cancer in women by 49% and 65%, respectively.^{3,5} However these agents only reduce rates of estrogen receptor-positive (ER+) breast cancer.³ SERMs have side effects, including increased risk of venous thromboembolism, endometrial cancer, stroke, irregular menses, hot flashes, bone loss, and cataracts; all of these have been barriers to widespread use of SERMs. While AIs do not have serious toxic effects, significant moderate side effects including myalgia, arthralgia, and osteoporosis are a barrier to use.⁴ The undesirable side effects of SERMs and the fact that tamoxifen is the only option for premenopausal women make these drugs less utilized, despite their efficacy. Though AIs have a better side effect profile and higher degree of breast cancer reduction than SERMs, these drugs are only effective in postmenopausal women; as with SERMs, AIs are only beneficial in preventing ER+ tumors. With limited choices for premenopausal women, side effects that are a barrier to use, and no current options for estrogen receptor-negative (ER-) cancers, investigation of additional options for chemoprevention are warranted.

One potential chemoprevention agent is vitamin D. Vitamin D is safe with few side effects, and studies support its anticancer properties.^{6,8} However, there is a paucity of clinical trials of vitamin D for breast cancer prevention. In a randomized, double-blind, 3-arm, placebo-controlled trial of calcium and vitamin D (1100 IU) in postmenopausal women, the incidence of all cancer was lower (after 4 years of treatment) in women taking both calcium and vitamin D⁹ with a trend toward fewer breast cancers. In another randomized, placebo-controlled trial evaluating only 400 international units (IU) of vitamin D daily

Introduction

Approximately 12% of women will develop breast cancer in their

FIGURE 1. Randomization scheme

for postmenopausal women, there was no difference in breast cancer incidence between the treatment and placebo groups.¹⁰ This study included over 36,000 women followed for an average of 7 years. The low dose of vitamin D in this study may have led to the negative findings.

Logistical barriers to studying the effect of any cancer-prevention agent include the large sample size and long duration of follow-up required to demonstrate efficacy. The use of cancer biomarkers, though, can circumvent these barriers. Biomarkers that are measurable and modifiable with treatment, with modification associated with change in cancer risk, are the most useful as intermediate endpoints.

Mammographic density (MD), cellular atypia, cellular proliferation, and insulin-like growth factor (IGF-1) are potentially useful intermediate biomarkers for breast cancer. MD has been confirmed as an independent risk factor for development of breast cancer.¹¹⁻¹⁴ Women with MD of at least 75% are 4 to 6 times more likely than women with MD of <10% to develop breast cancer.¹⁵ Cellular atypia and proliferation are also strongly linked to breast cancer development. An association between atypia and the development of breast cancer is well established.¹⁶ Ki-67, a nuclear protein that serves as a marker for cellular proliferation, is elevated in proliferating tissue.^{17,18} Elevated levels of IGF1 are also linked to risk for breast cancer. IGF-1 has a role in the growth of breast epithelial cells¹⁹ and the binding of IGF-1 to its receptor induces cellular proliferation, differentiation, and apoptosis.²⁰

Given the strong biological data supporting a possible preventive effect of vitamin D, this study was designed to determine the effect of 2000 IU of vitamin D on specific breast cancer biomarkers when taken daily for 1 year by premenopausal women. This manuscript will focus on the methods, recruitment, accrual, and baseline characteristics of participants of the trial.

Methods

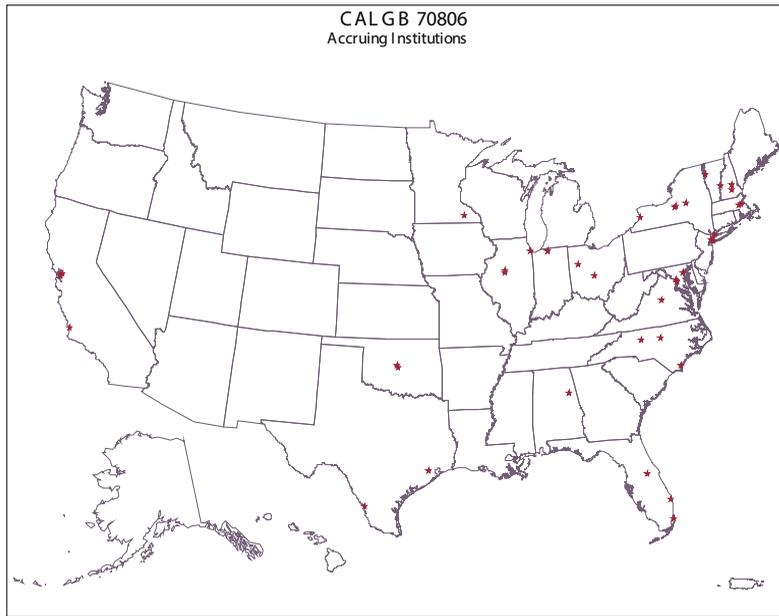
Cancer and Leukemia Group B (CALGB) 70806 is a randomized (1:1) double-blinded, placebo-controlled trial evaluating the

TABLE 1. Baseline Characteristics of Participants

Characteristic	Number N = 300 (%)
Age (yr)	
Range	22 - 59
Mean (SD)	42.6 (6.4)
Race	
African American	35 (11.7)
American Indian	1 (0.3)
Asian	14 (4.7)
Caucasian	238 (79.3)
Native Hawaiian	2 (0.7)
None Reported	5 (1.7)
More than one Race	5 (1.7)
Ethnicity	
Hispanic or Latino	42 (14)
Non-Hispanic	253 (84.3)
Unknown	5 (1.7)
Geographic Location	
Northeast	119 (39.6)
Midwest	42 (14.0)
South	114 (38.0)
West	25 (8.3)
BMI	
Range	15.1 - 53.6
Mean (SD)	27.0 (5.7)
Vitamin D Level	
<30 ng/mL	186 (62)
≥30 ng/mL	114 (38)
Range	4 - 72
Mean (SD)	26.6 (11.7)

effects of vitamin D taken for 1 year on several breast cancer biomarkers (breast density, cellular atypia, proliferation, and IGF-1) in premenopausal women with elevated breast density. CALGB is now part of the Alliance for Clinical Trials in Oncology (Alliance). Study participants were randomized to receive either vitamin D (2000 IU) or placebo daily for 12 months. The randomization scheme is shown in [Figure 1](#). Participants were stratified according to vitamin D levels (insufficient [<30 ng/mL] vs sufficient [≥ 30 ng/mL]) and random periareolar fine needle aspiration (RPFNA) status (institutions that performed the procedure vs those that did not).

FIGURE 2. Map of the United States showing recruitment sites of study participants with the location of each institution represented by a star.



and documentation of at least 5 samples with adequate cellularity.

Data collection was conducted by the Alliance Statistics and Data Center. Data quality was ensured by the Alliance Statistics and Data Center and by the study chairperson following Alliance policies.

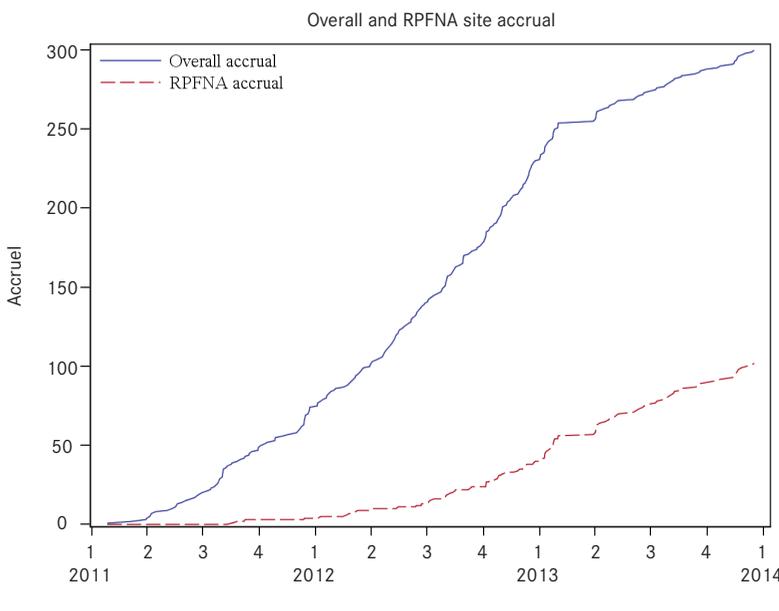
Eligibility Criteria: Women were eligible if they were premenopausal, had regular menstrual cycles (at least 4 cycles in the preceding 6 months), were 55 years of age or younger, and had a breast density of at least 25% (scattered fibroglandular density or greater).

Women were excluded if they had a history of breast, including ductal carcinoma in situ (DCIS) or ovarian cancer, breast implants or breast reduction surgery, serum calcium level >10.5 mg/dL, history of hyperparathyroid disease (or any disorder of calcium metabolism) requiring intervention in the past 5 years, history of kidney stones, 2 or more bone fractures in the past 5 years, diagnosis of osteoporosis requiring treatment, use of vitamin D supplements >400 IU/day, hormone replacement therapy tamoxifen or raloxifene, or concurrent participation in a breast cancer chemoprevention trial. Supplementation with additional vitamin D was an exclusion for participation. Medications were reviewed by research staff at each study visit.

Women using topical estrogens or hormonal contraceptives were eligible as long as they had been using the contraceptive more than 4 months prior to enrollment. Those on vitamin D therapy at a dose of 400 IU/day or less were also eligible as long as they had been on that dose for more than 6 months.

Enrollment: The study was open to institutions that were members of the Alliance and Community Clinical Oncology Program (CCOP) Network, and was available to other treating sites through the Cancer Trials Support Unit (CTSU). Institutional review board (IRB) approval was obtained at each participating institution, and informed consent

FIGURE 3. Rate of overall accrual and accrual for RPFNA participants



Prior to enrolling participants, institutions decided whether they would perform RPFNA, and RPFNA was performed on all participants enrolling at that particular institution. RPFNA was only performed at institutions with an experienced provider

was obtained from each study participant prior to enrollment.

Study Participation: Women were randomized to take vitamin D or placebo for 12 months starting within 14 days after

randomization. At baseline, women were examined, completed several questionnaires, and had blood drawn. They were contacted by study personnel for side effects at 3, 6, and 9 months. At completion of the study, women were examined and had blood drawn. Questionnaires at baseline included medical screening, aspirin use, diet, sunlight exposure, and physical activity questionnaires. Mammograms, serum 25-OH vitamin D and IGF-1, and RPFNA for cellular atypia and Ki-67 were obtained prior to initiating study medication and again at 12 months. Serum 25-OH vitamin D and IGF-1 levels were drawn and stored at -80° F centrally.

Cellular atypia and Ki-67 were evaluated only in participants enrolled at RPFNA-performing institutions. Aspirations were performed at 2 separate locations for each breast and samples from each participant were pooled, placed into cytolyte and sent to the University of Vermont for processing and analysis in the Department of Pathology's CLIA-approved cytopathology laboratory. Tissue samples obtained by RPFNA were evaluated in a blinded fashion by a single pathologist to assess atypia, and stained for Ki-67 (to evaluate proliferation).

Digital mammograms and reports were collected and stored at Imaging and Radiation Oncology Core (IROC) for MD determination. MD will be determined by three methods. The Breast Imaging-Reporting and Data System (BIRAD) density category will be abstracted from the mammogram report. MD will also be evaluated using both the semi-automated Boyd method¹¹ and a fully automated program.²¹⁻²³

Reported results are based on a dataset locked on August 21, 2014.

Statistical Analysis: The primary outcome for this trial is change in MD between the baseline and 12-month mammograms using the continuous thresholding method described by Boyd et al.²⁴ With 285 eligible women with data points at both baseline and 12 months, a 2-sample t-test with a 2-sided $\alpha=0.05$ will have 92% power to detect a standardized effect size of 0.4 in change of MD between 2 arms. With 250 eligible women with data points at both baseline and 12 months, a 2-sample t-test with a 2-sided $\alpha=0.05$ will have 88% power to detect a standardized effect size of 0.4 standard deviation change of MD between 2 arms.

Secondary outcomes include changes in atypia, cellular proliferation (measured by Ki-67 levels), and serum IGF-1. Changes in secondary outcomes (between baseline and 12 months) will be compared between the 2 arms using the same analysis methods as for the primary outcome.

For tissue biomarkers (atypia and Ki67), a sample size of 100 women provides a 2-sample t-test with 2-sided $\alpha = 0.05$ to have 85% power to detect a standardized effect size of 0.6 in change between 2 arms.

Correlations between change in biomarkers (MD, IGF1, atypia, and Ki67) with each other and with change in vitamin D levels will be addressed with simple linear regression analyses.

Multiple linear regression analyses will be conducted to relate the changes in MD or vitamin D level with changes in molecular markers and other predictors.

RESULTS

Participant Accrual: The study was activated in October 2010. The first patient was enrolled in January 2011 and the last in December 2013 and completed participation in January 2015. Three hundred participants were enrolled at 41 different institutions in the United States. RPFNA was performed at 14 different institutions with a total of 102 women undergoing the procedure. The geographic distribution of participating institutions is shown in **Figure 2**. The rate of accrual for overall participants as well as for RPFNA participants is shown in **Figure 3**.

The original sample size for this study was 250 patients, with plans for 100 participants to undergo RPFNA. The study reached the accrual goal of 250 patients at the beginning of February 2013, but fewer than 50 patients had participated in the RPFNA portion of the study. Study enrollment remained open only at institutions performing RPFNA until the goal was met. Sample size was increased to 300 participants, of whom 100 would undergo RPFNA.

Participant Characteristics: The mean age of participants was 42.6 years. Patients had a mean BMI of 27.0 (range 15.1-53.6). The mean vitamin D level was 26.6 ng/mL; the majority (62%) were vitamin D deficient (<30 ng/mL) at enrollment.

There were wide geographic and ethnic distributions among participants. The highest numbers of participants were recruited from the Northeast and South, accounting for 39.6% and 38.0% of the total sample, respectively. There was also ethnic diversity, as 14% and 11.7% of participants, respectively, self-reported as Hispanic and African American. Baseline demographic characteristics of participants are shown in **Table 1**.

Conclusions

This study represents one of the first chemoprevention trials conducted in the cooperative group setting with a focus on intermediate biomarkers of breast cancer risk. Clearly, this type of study can be accomplished in both academic and community settings with fairly short accrual time (23 months). The study has accrued an ethnically and geographically diverse population sample. The diversity of this sample gives strength to the potential for generalizability of the results.

The design of this chemoprevention trial, focusing on several key biomarkers, is novel. The companion tissue study was carefully supervised; only sites demonstrating proficiency were allowed to open it. It was anticipated that accrual at sites participating in RPFNA would be slower. Nonetheless, with the rapid accrual at non-RPFNA sites, it was necessary to close the study temporarily and re-open only at sites performing RPFNA. Temporary closures can result in slow accrual when

the study is re-opened, however, this did not happen. The last 50 participants were recruited in less time than originally projected. All accrual goals were met, with a total sample size greater than initially intended.

This study was carried out in premenopausal women and at a higher dose of vitamin D than in prior studies. Information obtained from this study will provide necessary data regarding the justification for a larger trial evaluating the effect of vitamin D on breast cancer development. Information on vitamin D levels at baseline and follow up will be reported at a later date, along with reporting of the primary outcome.

Acknowledgements: The authors are grateful to the patients who consented to participate in these clinical trials and the families who supported them. We wish to acknowledge the accrual of patients to this study by ECOG-ACRIN (supported by CA180820 and CA21115) and SWOG (supported by 1U10CA180888). The following institutions participated in this study:

Bay Area Tumor Institute NCORP, Oakland, CA, supported by Jon M. Greif, UG1CA189817

Dana-Farber/Partners CancerCare, Boston, MA, supported by Harold J. Burstein, U10CA180867

Dartmouth Medical School-Norris Cotton Cancer Center, Lebanon, NH, Konstantin Dragnev, supported by CA04326

Duke Cancer Institute, Duke University Medical Center, Durham, NC, supported by Jeffrey Crawford, U10CA180857

Florida Hospital Orlando, Orlando, FL, supported by Lee Zehngbot

Heartland Cancer Research NCORP, Decatur, IL, supported by James L. Wade III, UG1CA189830

Hematology-Oncology Associates of CNY CCOP, Syracuse, NY, Jeffrey Kirshner, supported by CA45389

Illinois Oncology Research Association, Peoria, IL, John W. Kugler, supported by CA35113

Mayo Clinic, Rochester, MN, supported by Steven R. Alberts, U10CA180790

Medstar Georgetown University Medical Center, Washington, DC, Bruce Cheson, supported by CA77597

Mount Sinai Medical Center, Miami, FL, Michael A. Schwartz, supported by CA45564

New Hampshire Oncology-Hematology PA, Concord, NH, Douglas J. Weckstein

Northern Indiana Cancer Research Consortium CCOP, South Bend, IN, Rafat Ansari, supported by CA86726

Queens Hospital Center, Jamaica, NY, supported by Mary Kemeny

Roswell Park Cancer Institute, Buffalo, NY, supported by Ellis G. Levine, U10CA180866

Southeast Cancer Consortium-Upstate NCORP, Winston-Salem, NC, supported by James N. Atkins, UG1CA189858

State University of New York Upstate Medical University, Syracuse, NY, Stephen L. Graziano, supported by CA21060

The Ohio State University, Columbus, OH, supported by Richard M. Goldberg, U10CA180850

University of California at San Diego Moores Cancer Center, San Diego, CA, Barbara A. Parker, supported by CA11789

University of North Carolina, Chapel Hill, NC, supported by Lisa A. Carey, U10CA180838

University of Oklahoma, Oklahoma City, OK, supported by Robert S. Mannel, U10CA180798

University of Texas Southwestern Medical Center, Dallas, TX, supported by Joan Schiller, U10CA180870

University of Vermont, Burlington, VT, Steven M. Grunberg, supported by CA77406

Walter Reed Army Medical Center, Washington, DC, David C. Van Echo, supported by CA26806

Declaration of conflict: The authors declare that there is no conflict of interest.

Affiliations: Ogheneruona Apoe, MD, and Marie Wood, MD, are with the Division of Hematology and Oncology, Department of Medicine, University of Vermont, Burlington VT; Sin-Ho Jung, PhD, is with Alliance Statistics and Data Center, Duke University, Durham, NC; Heshan Liu and Drew K Seisler are with Alliance Statistics and Data Center, Mayo Clinic, Rochester, MN; Jayne Charlamb, MD, is with State University of New York Upstate Medical University, Syracuse, NY; Patricia Zekan, MD, is with Novant Health Forsyth Medical Center, Winston-Salem, NC; Lili X. Wang, MD, is with Contra Costa Regional Medical Center, Martinez, CA; Gary W. Unzeitig, MD, is with Doctor's Hospital of Laredo, Laredo, TX; Judy Garber, MD, is with Dana-Farber/Partners CancerCare, Boston, MA; and James Marshall, PhD, is with Roswell Park Cancer Institute, Buffalo, NY.

Corresponding author: Marie Wood, Division of Hematology and Oncology, University of Vermont, 89 Beaumont Ave, Given E-214, Burlington VT 05405. Phone: (802) 656-5487, Fax: (802) 656-5493. E-mail: marie.wood@uvm.edu

Support: Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number UG1CA189823 (to the Alliance for Clinical Trials in Oncology), U10CA21060, U10CA180867, U10CA180866, UG1CA189817, and UG1CA189858. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Also supported in part by funds from 5R21CA137650-2 (PI M. Wood).

Clinical Trials.gov Identifier: NCT01224678

REFERENCES

1. Siegel, RL, Miller KD, and Jemal A. *CA Cancer J Clin.* 2015;65(1):5-29. doi: 10.3322/caac.21254.
2. Tabár L, Fagerberg CJ, Gad A, et al. Reduction in mortality from breast cancer after mass screening with mammography.

Randomised trial from the Breast Cancer Screening Working Group of the Swedish National Board of Health and Welfare. *Lancet*. 1985;1(8433):829-832.

3. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst*. 1998;90(18):1371-1388.

4. Goss PE, Ingle JN, Alés-Martínez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med*. 2011;364(25):2381-2391. doi: 10.1056/NEJMoa1103507.

5. Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. *Lancet*. 2014;383(9922):1041-1048. doi: 10.1016/S0140-6736(13)62292-8.

6. Feldman D, Krishnan AV, Swami S, et al. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer*. 2014;14(5):342-357. doi: 10.1038/nrc3691.

7. Krishnan AV, Swami S, Feldman D. Equivalent anticancer activities of dietary vitamin D and calcitriol in an animal model of breast cancer: importance of mammary CYP27B1 for treatment and prevention. *J Steroid Biochem Mol Biol*. 2013;136:289-295. doi: 10.1016/j.jsbmb.2012.08.005.

8. Chakraborti, CK. Vitamin D as a promising anticancer agent. *Indian J Pharmacol*. 2011;43(2):113-120. doi: 10.4103/0253-7613.77335.

9. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr*. 2007;85(6):1586-1591.

10. Chlebowski RT, Johnson KC, Kooperberg C, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Inst*. 2008;100(22):1581-1591. doi: 10.1093/jnci/djn360.

11. Boyd NF, Martin LJ, Bronskill M, Yaffe MJ, Duric N, Minkin S. Breast tissue composition and susceptibility to breast cancer. *J Natl Cancer Inst*. 2010;102(16):1224-1237. doi: 10.1093/jnci/djq239.

12. Shepherd JA, Kerlikowske K, Ma L, et al. Volume of mammographic density and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2011;20(7):1473-1482. doi: 10.1158/1055-9965.EPI-10-1150.

13. Pinto Pereira SM, McCormack VA, Hipwell JH, et al. Localized fibroglandular tissue as a predictor of future tumor location within the breast. *Cancer Epidemiol Biomarkers Prev*. 2011;20(8):1718-1725. doi: 10.1158/1055-9965.EPI-11-0423.

14. Pettersson A, Graff RE, Ursin G, et al. Mammographic density phenotypes and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst*. 2014;106(5). pii: dju078. doi: 10.1093/jnci/dju078.

15. Boyd, N.F., *Mammographic density and risk of breast cancer*. *Am Soc Clin Oncol Educ Book*. 2013. doi: 10.1200/EdBook_

AM.2013.33.e57.

16. Neal L, Sandhu NP, Hieken TJ, et al. Diagnosis and management of benign, atypical, and indeterminate breast lesions detected on core needle biopsy. *Mayo Clin Proc*. 2014;89(4):536-547. doi: 10.1016/j.mayocp.2014.02.004.

17. Urruticoechea A1, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol*. 2005;23(28):7212-7220.

18. Santisteban M, Reynolds C, Barr Fritcher EG, et al. Ki67: a time-varying biomarker of risk of breast cancer in atypical hyperplasia. *Breast Cancer Res Treat*. 2010;121(2):431-437. doi: 10.1007/s10549-009-0534-7.

19. Uray IP, Brown PH. Chemoprevention of hormone receptor-negative breast cancer: new approaches needed. *Recent Results Cancer Res*. 2011;188:147-162. doi: 10.1007/978-3-642-10858-7_13.

20. Tas F, Karabulut S, Bilgin E, Tastekin D, Duranyildiz D. Clinical significance of serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) in patients with breast cancer. *Tumour Biol*. 2014;35(9):9303-9309. doi: 10.1007/s13277-014-2224-2.

21. Keller BM, Chen J, Conant EF, Kontos D. Breast density and parenchymal texture measures as potential risk factors for Estrogen-Receptor positive breast cancer. *Proc SPIE Int Soc Opt Eng*. 2014;9035:90351D.

22. Keller B, Nathan D, Wang Y, et al. Adaptive multi-cluster fuzzy C-means segmentation of breast parenchymal tissue in digital mammography. *Med Image Comput Comput Assist Interv*. 2011;14(Pt 3):562-569.

23. Kontos D1, Ikejimba LC, Bakic PR, Troxel AB, Conant EF, Maidment AD. Analysis of parenchymal texture with digital breast tomosynthesis: comparison with digital mammography and implications for cancer risk assessment. *Radiology*. 2011;261(1):80-91. doi: 10.1148/radiol.11100966.

24. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. *J Natl Cancer Inst*. 1995;87(9):670-675.