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The Effect of Black Cohosh on Ki67 expression and Tumor Volume: A Pilot Study of Ductal Carcinoma in Situ Patients

Amelia A. Trant, MD[1], Anees Chagpar, MD, MBA, MPH, FACS, FRCS(C)[1], Wei Wei, PhD[1], Veronique Neumeister, MD[1], David Rimm, MD, PhD[1], Karen Stavris, RN[1], Bonnie Lurie, RN[1], Courtney Frederick, RN[1], Liva Andrejeva, MD[1], Madhavi Raghu, MD[1], Brigid Killelea, MD, MPH, FACS[1], Nina Horowitz, MD, FACS[1], Donald Lannin, MD[1], Elspeth Knill-Selby, APRN[1], Tracy Sturrock, APRN[1], and Erin Hofstatter, MD[1]

Abstract

Background: Black cohosh (BC) (*Cimicifuga racemosa*) may prevent and treat breast cancer through anti-proliferative, pro-apoptotic, anti-estrogenic, and anti-inflammatory effects. This study sought to evaluate the effect of BC on tumor cellular proliferation, measured by Ki67 expression, in a pre-operative window trial of ductal carcinoma in situ (DCIS) patients. **Methods:** Patients were treated pre-operatively for 2 to 6 weeks with BC extract. Eligible subjects were those who had DCIS on core biopsy. Ki67 was measured using automated quantitative immunofluorescence (AQUA) pre/post-operatively. Ki67, tumor volume, and hormone changes were assessed with 2-sided Wilcoxon signed-rank tests, $\alpha = .05$. **Results:** Thirty-one patients were treated for an average of 24.5 days (median 25; range 15-36). Ki67 decreased non-significantly (n = 26; $P = .20$; median pre-treatment 1280, post-treatment 859; range pre-treatment 175-7438, post-treatment 162-3370). Tumor volume, estradiol, and FSH did not change significantly. No grade 3 or 4 adverse events were reported. **Conclusions:** BC use showed no significant impact on cellular proliferation, tumor volume, or invasive disease upgrade rates in DCIS patients. It was well-tolerated, with no observed significant toxicities. Further study is needed to elucidate BC's role in breast cancer treatment and prevention.
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Keywords

black cohosh, cancer prevention, pilot study, window trial, breast cancer

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Introduction

Breast cancer is a major public health issue, given the vast number of women who are affected by this disease. Recent statistics indicate that 13% of American women will be diagnosed in their lifetimes.[1] Treatment and prevention of breast cancer has focused on estrogen-receptor (ER) blockade.[2-4] However, the 20% to 30% of cancers that are ER-negative, prove to be among the most aggressive and are not susceptible to estrogen-targeting strategies. Furthermore, many women opt to discontinue adjuvant hormonal therapy or opt against initiation of breast cancer chemoprevention, due to intolerable side effects and potential health risks.[2-4] These data underscore the need to identify novel approaches and new agents for the prevention and treatment of breast cancer.

Recent preclinical, clinical, and epidemiologic evidence suggests that black cohosh (*Cimicifuga racemosa*) (BC)

[1]Yale School of Medicine, New Haven, CT, USA

Corresponding Author:
Erin Hofstatter, Yale School of Medicine, 333 Cedar St., PO Box 208032 New Haven, CT 05620, USA.
Email: erin.hofstatter@yale.edu

may be a potential agent for breast cancer prevention and treatment.[5] This centuries-old medicinal herb is used primarily for relief from menopausal symptoms today. Several clinical trials have studied its efficacy against hot flashes.[6] The active ingredients in BC preparations appear to be triterpene glycosides.[5,7] Though the exact mechanisms of action remain unclear, recent preclinical data suggest triterpenes may prevent and treat breast cancer, through anti-proliferative, pro-apoptotic, anti-estrogenic, anti-inflammatory, and anti-oxidant effects.[7-11] Epidemiologic data also show promising results; a retrospective case-control study showed that the use of BC had a significant protective effect against the development of breast cancer (adjusted OR 0.39, CI: 0.22-0.70), and a German study demonstrated prolonged disease-free survival in breast cancer patients taking BC.[12,13] Additionally, it has been used in several clinical trials on hot flashes without safety or tolerability concerns.[14-16]

Testing new agents in breast cancer, particularly in the DCIS or preventive setting, is challenging because clinical studies using incidence or recurrence as endpoints are costly and time-consuming. An alternative approach to a large randomized controlled trial is a study that examines the new agent for preliminary evidence of efficacy via sampling of breast tissue, using surrogate biomarkers as study endpoints. A common approach is a pre-surgical "window" model, where women who are awaiting lumpectomy or mastectomy as the standard of care are enrolled in a trial. This way, pre-and post-drug tissue can be examined to demonstrate efficacy while minimizing the need for extra biopsies. This approach has been successfully employed with other candidate agents in early-stage breast cancer.[17-19] Pre-surgical window trials often use surrogate biomarkers, which are measurable, reliable, and highly associated with breast cancer risk; common markers include: Ki67, a marker of cellular proliferation, and change in lesion size, a biomarker for tumor response and outcomes assessment.[20,21]

Based on these observations, we hypothesized that BC has strong potential to be an effective treatment and prevention agent for breast cancer and that preliminary evidence of its efficacy could be demonstrated in a pre-operative window trial in women with ductal carcinoma in situ (DCIS). Specifically, this study aimed to assess the impact of BC treatment on Ki67 levels as a marker of tumor cell proliferation and tumor aggressiveness, and tumor volume as a measure of tumor response, in patients with Ductal Carcinoma in Situ (DCIS) over a pre-operative window of 2 to 6 weeks.

Methods

Patient Screening and Recruitment: This single-site study was conducted at Smilow Cancer Hospital; the study was approved by the Institutional Review Board at Yale University and written informed consent was provided by all patients. The study ID number is HIC#1205010204 and it was approved on May 30, 2012 recruiting began on June 1, 2012. Pre and post-menopausal women with newly diagnosed and core biopsy-confirmed Ductal Carcinoma in Situ (DCIS) were eligible for the study. Participants had to be willing to commit to 2 to 6 weeks of treatment prior to their breast surgery. The study was introduced to potential subjects by their surgeons at their initial surgical consult. Patients who were under the age of 18, were pregnant or nursing within the preceding 6 months, were deemed not capable of providing consent, or had recently taken any exogenous hormonal therapy or a substance known to interact with BC were ineligible.

BC and Surgical Treatment: Participants were given a course of commercial standardized isopropanolic BC, 20 mg orally twice per day for 2 to 6 weeks prior to their surgery. This dose was selected based on previous clinical studies and manufacturer recommendations.[22,23] Extracts of BC are standardized to 26-deoxyactein content, such that each 20 mg tablet contains 2.5 mg of 26-deoxyactein. The final dose of BC was taken the day prior to surgery. Subjects underwent a definitive excision of their DCIS consistent with either standard of care mastectomy or breast-conserving lumpectomy. The choice of procedure was made irrespective of this study.

Safety Measures: Baseline laboratory studies were obtained on all patients, including complete blood count, comprehensive metabolic panel, and hormone panel including estradiol and follicular stimulating hormone (FSH). Study medication compliance, post-treatment laboratory studies, and assessment of tolerability, toxicity, and adverse events were collected on the day of surgery. Study assessments included a history and physical, routine blood work including liver function tests, and toxicity assessment. A safety phone call was made approximately 30 days after surgery to assess adverse events. All adverse events, whether observed by the physician or reported by the patient, occurring during the active portion of therapy, or up to 30 days after the last dose of treatment were graded by a numerical score according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE). Labs, history and physical exam, and adverse events were reviewed by the treating physician and principal investigator of the study to ensure that all results were within a normal range. Due to concern for the potential phyto-estrogenic activity of BC, hormonal levels for post-menopausal women were compared pre- and post-treatment to elucidate the impact of BC on these measures.[10,11,24-26] Data from pre-menopausal women could not be compared as the bloodwork was not taken at the same time point in their menstrual cycles, but the blood work was assessed to ensure that their levels remained in the normal range.

Imaging: Patients had either a mammogram, ultrasound, or MRI as part of normal screening procedures prior to study enrollment. This imaging was used to estimate

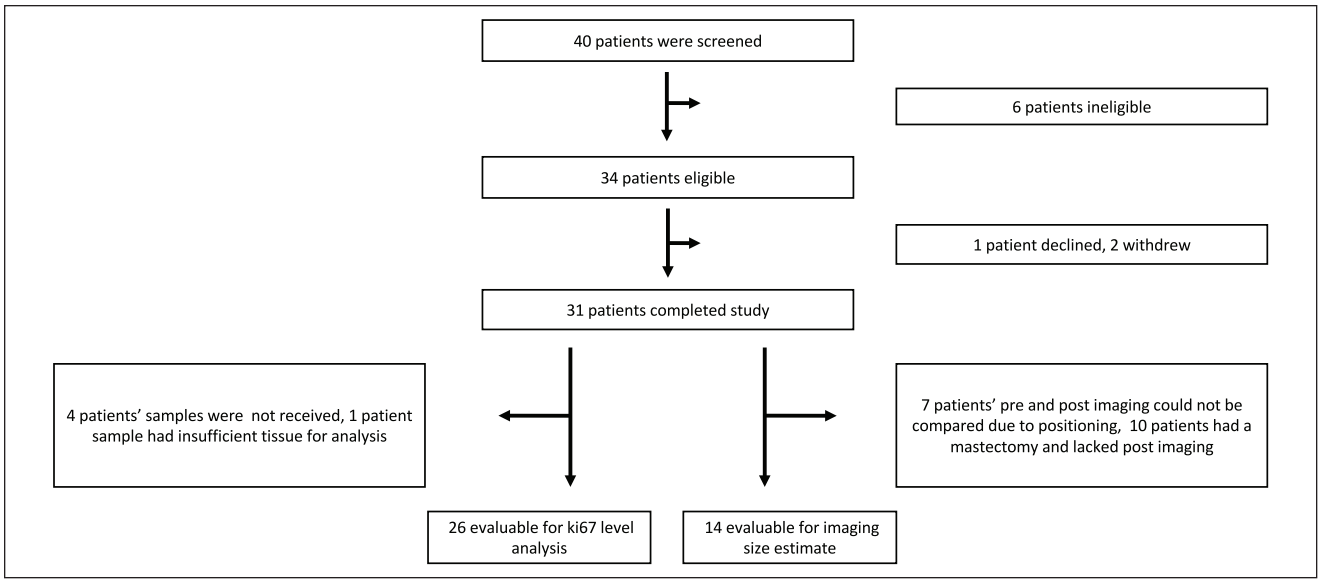

Figure 1. Study recruitment, participation, and completion.

pre-treatment tumor volume. While baseline standard of care (SOC) breast imaging was obtained for all patients, only those participants who chose to undergo a breast conservation procedure (ie, lumpectomy) for their DCIS had additional SOC imaging on the day of surgery. A breast radiologist read and estimated the size of the tumors in pre- and post-imaging for these patients. The radiologist measured the largest diameter of the calcifications or the mass and reported height, width, and length. These measurements were used to calculate volume. The volume measurements were validated by a second radiologist to reduce any potential bias.

Tissue Sample: A SOC core biopsy confirming the diagnosis of DCIS was taken prior to study enrollment. This tissue was subject to standard institutional pathological review. A sample of the paraffin-embedded tissue was obtained for later research analysis. The breast tissue obtained from surgery was subject to the same procedures. Staining was performed using the DAKO MIB1 Ki67 antibody at previously optimized and standardized conditions.[27,28] Ki67 expression levels were measured using automated quantitative immunofluorescence (AQUA). Only areas with DCIS as assessed by a pathologist were included for Ki67 scoring. AQUA is a set of algorithms that measure immunofluorescence and therefore expression of in situ proteins including Ki67 as previously described.[29] This analysis was performed over multiple fields of view on each sample to increase accuracy; the average AQUA score across all was used as the Ki67 score.[28] AQUA calculated Ki67 values based on individual pixel intensity of the immunofluorescence; the values from each field of view are averaged. Then the averages of all the fields of view taken from a given sample are averaged for a final Ki67 value. Therefore, the Ki67 values calculated have units of average pixel intensity per sample.

Statistical Considerations: A sample size of 22 patients, assuming a 10% drop-out rate, was estimated to achieve 91% power if the mean of paired differences (pre- vs post-marker values) is 0.8 standard deviation difference from 0 at a 2-sided significance level at .05 using Wilcoxon signed-rank test. Changes in Ki67 expression, tumor volume, and hormone levels were each assessed with paired Wilcoxon signed-rank tests and a significance level of .05. The Wilcoxon signed-rank test was chosen for this analysis because it is the non-parametric version of the paired t-test. It assesses the medians of the 2 sets of samples rather than the mean, which makes it more robust in small sample sizes and against non-normal distributions and potential outliers.[30] R version 3.4.4 and R Studio version 1.1.442 were used for all statistical analyses.

Results

Enrollment and Patient Characteristics: Out of the 34 eligible patients invited to participate, 31 completed the study and received an average of 24.5 days (median 25; range 15-36) of BC. Due to missing data, 26 were included in the Ki67 analysis, and 14 of the 21 patients who underwent lumpectomies were included in the tumor volume calculations (Figure 1). All patients were females; the median age was 58 years (range 34-79) and the majority had hormone-positive disease (Table 1).

Tumor Volume: Among women undergoing lumpectomies, there was no significant change observed between

Table 1. Pathological Data and Patient Demographics.

Patient characteristics	Participants (%)
DCIS grade pre-treatment	
Grade 1	6 (19)
Grade 2	12 (39)
Grade 3	13 (42)
DCIS grade post-treatment	
Grade 1	3 (10)
Grade 2	10 (32)
Grade 3	16 (52)
No remaining DCIS	2 (6)
DCIS subtype	
Comedo	7 (23)
Non-Comedo	24 (77)
Receptor status	
ER positive	23 (74)
PR positive	24 (77)
Missing data	1 (3)
Surgical procedure	
Mastectomy	10 (32)
Lumpectomy	21 (68)
Final diagnosis post-treatment	
Hyperplasia	2 (6)
DCIS	18 (58)
Microinvasive	3 (10)
Invasive	8 (26)
Menopausal status	
Premenopausal	12 (39)
Postmenopausal	19 (61)
Age	
<50 y old	6 (19)
50-59 y old	10 (32)
60-70 y old	9 (29)
>70 y old	6 (19)
Number of days on treatment	
15-21 d	13 (42)
22-28 d	7 (23)
29-36 d	11 (35)

the average pre- and post- tumor volume (n = 14; $P = .33$; median pre-treatment 8.1 cm^3; median post-treatment 5.18 cm^3; range pre-treatment 0.063-60.76 cm^3; range post-treatment 0.042-56.42 cm^3) (Figure 3A and B).

Hormone Levels: There was an observed 51% decrease in estradiol levels of post-menopausal women after BC treatment, which was not statistically significant (n = 15; $W = 38$; $P = .60$; median pre-treatment 20; median post-treatment 19; range pre-treatment 1.5-428; range post-treatment 4.6-133). A pairwise comparison showed a non- significant 7% decrease in the average follicle-stimulating hormone (FSH) levels of the post-menopausal women after taking the BC treatment (n = 17; $W = 10$; $P = .04$; median pre-treatment 63; median post-treatment 62; range pre-treatment 31-170; range post-treatment 25-148); however, as the values remained in the normal range for post-menopausal women the change is not considered clinically significant, particularly in the context of unchanged estradiol levels over the same time period. Evaluation of hormonal studies was limited in premenopausal women given the short treatment window in relation to a typical menstrual cycle, though FSH and estradiol values remained in a normal range for all premenopausal patients.

Final Diagnoses: All patients who participated in the study had an initial biopsy which confirmed the presence of DCIS. After surgery, 6% of patients had no remaining DCIS but had some remaining hyperplasia, 10% had micro-invasive cancer, and 26% had invasive breast cancer in addition to their DCIS (Table 1). This upgrade rate is consistent with the published literature; for example, a meta-analysis by Brennan et al[31] showed that invasive cancer is found at excision after an initial biopsy showing DCIS in 26% of cases; high grade and large tumor size increased the risk of understaging.

Adverse Events: A comprehensive list of related adverse events is reported in Table 2. The most common adverse event was diarrhea affecting 2 participants (6.4% of all participants). All other adverse events affected only 1 participant and included heartburn/dyspepsia, breast fullness, knee pain, vaginal spotting, nausea, breast pain, and bloating (3.2% of participants for each side effect respectively). All events were grade 1 with the exception of the knee pain which was grade 2. Overall the treatment was considered to be well-tolerated; no patients discontinued treatment due to side effects. No Grade 3 or 4 adverse events were reported.

Discussion

To our knowledge, this is the first prospective window trial to examine the effect of BC in a cohort of pre-operative DCIS patients. The observed downward trend in Ki67 shows the potential for efficacy of BC in a breast cancer treatment or prevention setting. Notably, there was no observed change in tumor volume or postmenopausal hormone levels, and no observed increase in upgrade to invasive cancers as compared with published literature.[31] While there was an observed drop in post-menopausal estrogen levels, this finding was not statistically significant and some fluctuation in hormone levels is to be expected based on physiology as well as lab conditions; overall the pre and post –treatment median estradiol levels were similar at 20 and 19 respectively. In other studies, BC has not been shown to significantly impact hormone levels.[32] BC was well-tolerated in this study, and no patients discontinued treatment due to side effects. Taken together, these findings merit further investigation in a larger study of breast cancer patients with longer exposure to BC.

Our study has several strengths, including examination of a novel agent through the use of a well-established clinical

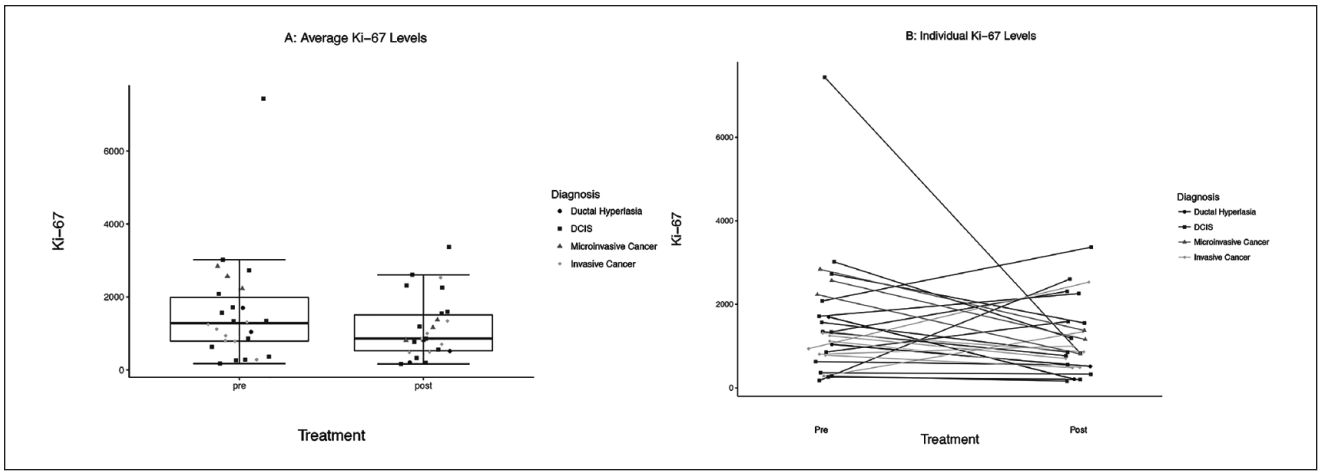

Figure 2. (A) Boxplot of Ki67 data. The mean pre-treatment Ki67 value was 1564 (standard deviation 1462) with a range of 175 to 7438. The mean post-treatment Ki67 value was 1157 (standard deviation 844) with a range of 162 to 3370. (B) Plot of matching individual patient's pre and post treatment Ki67 scores.

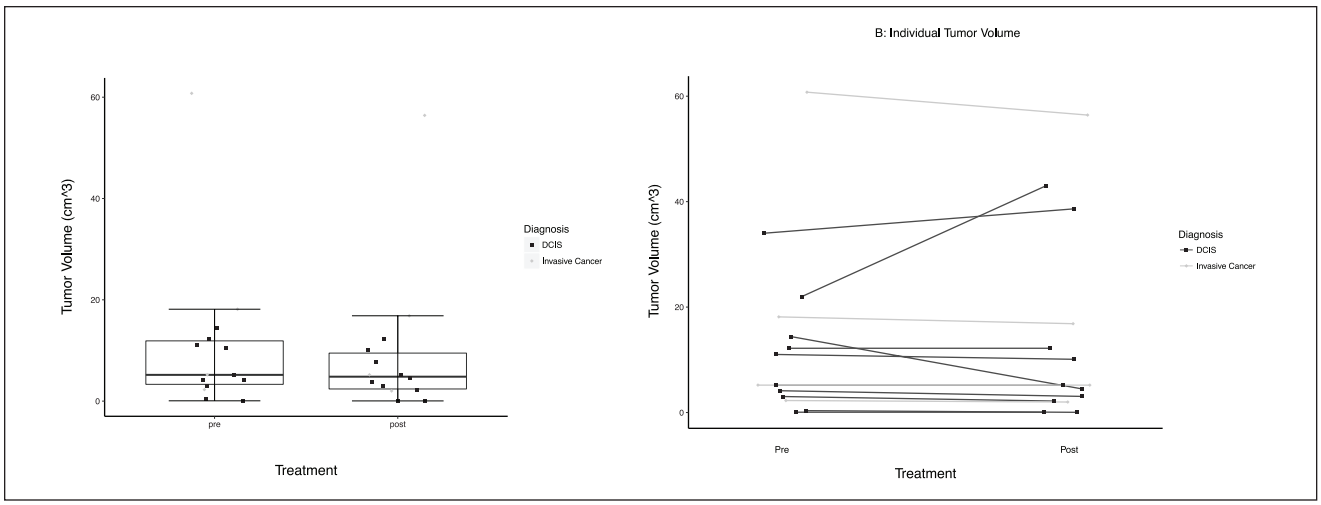

Figure 3. (A) boxplot of pre and post treatment tumor volume for patients who underwent lumpectomies. The mean pre-treatment tumor volume was 13.77 cm^3 (standard deviation 16.57 cm^3) range (0.063-60.76 cm^3). The mean post-treatment tumor volume was 14.24 cm^3 (standard deviation 18.21 cm^3) range (0.042-56.42 cm^3). (B) Plot of matching individual patient's pre and post treatment tumor volumes.

Table 2. Adverse Events Reported During Study and Follow Up.

Adverse event	Grade	Participants (%),	n = 31
Heartburn/dyspepsia	1	1	(3.2)
Diarrhea	1	2	(6.4)
Breast fullness	1	1	(3.2)
Pain-worsening pain in knees $L > R$	2	1	(3.2)
Vaginal spotting	1	1	(3.2)
Nausea	1	1	(3.2)
Pain bilateral breasts	1	1	(3.2)
Bloating	1	1	(3.2)

window trial design, use of a widely-applied surrogate biomarker such as Ki67 for determination of drug effect, and broad inclusion criteria with both pre and post-menopausal women to capture a representative sample of patients who may be seeking use of BC in a clinical setting. This study also has limitations. The study's small cohort and the relatively short treatment window are among its major limitations; the study design was intentionally set as such to limit any potential clinical risk from delay in definitive surgery, was modeled after other breast cancer window trials previously published, and was intended as a small pilot study. While common window-trial procedures were followed, tumor volume measurements could have been influenced by biopsy changes or imaging positioning.[33,34] An additional limitation is the surrogate use of Ki67 as a biomarker of tumor response to BC. While traditional Ki67 staining is widely viewed as an imprecise measure, for this reason we employed the use of the quantitative AQUA technique specifically to mitigate any error in measurement. Finally, this study was conducted at a single institution with a small group of patients, all of whom had DCIS; as such, our findings should be interpreted with caution, particularly for those patients with invasive breast cancer.

Conclusion

We observed that the use of BC in a pre-operative window trial setting demonstrated no significant change in breast cancer cellular proliferation, tumor volume, or invasive disease upgrade rates in women with DCIS. BC was well-tolerated, with no observed significant toxicities or changes in post-menopausal estrogen levels. Further study is needed to elucidate the role that BC may play in breast cancer treatment and prevention, given observed downward trends in Ki67.

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None

Author Contributions

Amelia A. Trant, MD; United States; data analysis, manuscript writing,
Anees Chagpar, MD, MBA, MPH, FACS, FRCS(C); United States; study design and recruitment, manuscript editing
Wei Wei, PhD; United States; Yale School of Medicine study design, data analysis
Veronique Neumeister, MD; United States; Indivumed Gro00up data analysis
David Rimm, MD, PhD; United States; data analysis, manuscript editing study design
Karen Stavris, RN; United States; study design and recruitment, manuscript editing
Bonnie Lurie, RN; United States; study design and recruitment, manuscript editing
Courtney Frederick, RN; United States; study design and recruitment, manuscript editing
Liva Andrejeva, MD; United States; radiology data analysis, manuscript editing
Madhavi Raghu, MD; United States; radiology data analysis, manuscript editing
Brigid Killelea, MD, MPH, FACS; United States; study design and recruitment, manuscript editing
Nina Horowitz, MD, FACS; United States; study design and recruitment, manuscript editing
Donald Lannin, MD; United States; study design and recruitment, manuscript editing
Elspeth Knill-Selby, APRN; United States; study design and recruitment, manuscript editing
Tracy Sturrock, APRN; United States; study design and recruitment, manuscript editing
Erin Hofstatter, MD; United States; Principal investigator

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Erin Hofstatter, MD is an employee at GlaxoSmithKline, the other authors report no conflict of interest

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ORCID iD

Amelia A. Trant https://orcid.org/0000-0002-9945-3696

References

1. American Cancer Society. Key statistics for breast cancer. American Cancer Society. 2015. Accessed April 13, 2022. https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html
2. Crew KD. Addressing barriers to uptake of breast cancer chemoprevention for patients and providers. *Am Soc Clin Oncol Educ Book*. 2015;e50-e58. doi:10.14694/EdBook_AM.2015.35.e50
3. Reimers LL, Sivasubramanian PS, Hershman D, et al. Breast cancer chemoprevention among high-risk women and those with ductal carcinoma in situ. *Breast J*. 2015;21:377-386.
4. Ropka ME, Keim J, Philbrick JT. Patient decisions about breast cancer chemoprevention: a systematic review and meta-analysis. *J Clin Oncol*. 2010;28:3090-3095.
5. National Institute of Health Office of Dietary Supplements. 2021. Accessed April 13, 2022. Black cohosh: fact sheet, http://ods.od.nih.gov/factsheets/blackcohosh
6. Mohapatra S, Iqubal A, Ansari MJ, et al. Benefits of black cohosh (Cimicifuga racemosa) for women health: an up-close and in-depth review. *Pharmaceuticals*. 2022;15:278.
7. Ruhlen RL, Sun GY, Sauter ER. Black cohosh: insights into its mechanism(s) of action. *Integr Med Insights*. 2008;3:21-32.

8. Einbond LS, Su T, Wu HA, et al. Gene expression analysis of the mechanisms whereby black cohosh inhibits human breast cancer cell growth. *Anticancer Res*. 2007;27:697-712.
9. Hostanska K, Nisslein T, Freudenstein J, Reichling J, Saller R. Cimicifuga racemosa extract inhibits proliferation of estrogen receptor-positive and negative human breast carcinoma cell lines by induction of apoptosis. *Breast Cancer Res Treat*. 2004;84:151-160.
10. Crone M, Hallman K, Lloyd V, et al. The antiestrogenic effects of black cohosh on BRCA1 and steroid receptors in breast cancer cells. *Breast Cancer (Dove Med Press)*. 2019;11:99-110.
11. Szmyd M, Lloyd V, Hallman K, et al. The effects of black cohosh on the regulation of estrogen receptor (ERalpha) and progesterone receptor (PR) in breast cancer cells. *Breast Cancer*. 2018;10:1-11.
12. Rebbeck TR, Troxel AB, Norman S, et al. A retrospective case-control study of the use of hormone-related supplements and association with breast cancer. *Int J Cancer*. 2007;120:1523-1528.
13. Henneicke-von Zepelin HH, Meden H, Kostev K, Schröder-Bernhardi D, Stammwitz U, Becher H. Isopropanolic black cohosh extract and recurrence-free survival after breast cancer. *Int J Clin Pharmacol Ther*. 2007;45:143-154.
14. National Center for Complementary and Alternative Medicine NIH Office of Dietary Supplements. Workshop on the safety of black cohosh in clinical studies. National Institutes of Health. 2004.
15. Loprinzi CL, Barton DL, Sloan JA, et al. Mayo Clinic and North Central Cancer Treatment Group hot flash studies: a 20-year experience. *Menopause*. 2008;15:655-660.
16. Shams T, Setia MS, Hemmings R, McCusker J, Sewitch M, Ciampi A. Efficacy of black cohosh-containing preparations on menopausal symptoms: a meta-analysis. *Altern Ther Health Med*. 2010;16:36-44.
17. Fabian CJ, Kimler BF, Mayo MS, Khan SA. Breast-tissue sampling for risk assessment and prevention. *Endocr Relat Cancer*. 2005;12:185-213.
18. Kalinsky K, Zheng T, Hibshoosh H, et al. Proteomic modulation in breast tumors after metformin exposure: results from a "window of opportunity" trial. *Clin Transl Oncol*. 2017;19:180-188.
19. Brown K, Rufini A. New concepts and challenges in the clinical translation of cancer preventive therapies: the role of pharmacodynamic biomarkers. *Ecancermedicalscience*. 2015;9:601.
20. Dowsett M, Bundred NJ, Decensi A, et al. Effect of raloxifene on breast cancer cell ki67 and apoptosis: a double-blind, placebo-controlled, randomized clinical trial in postmenopausal patients. *Cancer Epidemiol Biomarkers Prev*. 2001;10:961-966.
21. Miller M, Ottesen RA, Niland JC, Kruper L, Chen SL, Vito C. Tumor response ratio predicts overall survival in breast cancer patients treated with neoadjuvant chemotherapy. *Ann Surg Oncol*. 2014;21:3317-3323.
22. Pockaj BA, Gallagher JG, Loprinzi CL, et al. Phase III double-blind, randomized, placebo-controlled crossover trial of black cohosh in the management of hot flashes: NCCTG trial N01CC1. *J Clin Oncol*. 2006;24:2836-2841.
23. Briese V, Stammwitz U, Friede M, Henneicke-von Zepelin HH. Black cohosh with or without St. John's wort for symptom-specific climacteric treatment–results of a large-scale, controlled, observational study. *Maturitas*. 2007;57:405-414.
24. Rietjens IMCM, Louisse J, Beekmann K. The potential health effects of dietary phytoestrogens. *Br J Pharmacol*. 2017;174:1263-1280.
25. McKenna DJ, Jones K, Humphrey S, Hughes K. Black cohosh: efficacy, safety, and use in clinical and preclinical applications. *Altern Ther Health Med*. 2001;7:93-100.
26. Fritz H, Seely D, McGowan J, et al. Black cohosh and breast cancer: a systematic review. *Integr Cancer Ther*. 2014;13:12-29.
27. Cattoretti G, Becker MH, Key G, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol*. 1992;168:357-363.
28. Brown JR, DiGiovanna MP, Killelea B, Lannin DR, Rimm DL. Quantitative assessment Ki-67 score for prediction of response to neoadjuvant chemotherapy in breast cancer. *Lab Invest*. 2014;94:98-106.
29. Dolled-Filhart M, Gustavson M, Camp RL, Rimm DL, Tonkinson JL, Christiansen J. Automated analysis of tissue microarrays. *Methods Mol Biol*. 2010;664:151-162.
30. Direct S. Wilcoxon signed-rank test. 2021. Accessed April 13, 2022. https://www.sciencedirect.com/topics/mathematics/wilcoxon-signed-rank-test
31. Brennan ME, Turner RM, Ciatto S, et al. Ductal carcinoma in situ at core-needle biopsy: meta-analysis of underestimation and predictors of invasive breast cancer. *Radiology*. 2011;260:119-128.
32. Castelo-Branco C, Gambacciani M, Cano A, et al. Review & meta-analysis: isopropanolic black cohosh extract iCR for menopausal symptoms - an update on the evidence. *Climacteric*. 2021;24:109-119.
33. Huber S, Wagner M, Medl M, Czembirek H. Benign breast lesions: minimally invasive vacuum-assisted biopsy with 11-gauge needles patient acceptance and effect on follow-up imaging findings. *Radiology*. 2003;226:783-790.
34. Cortadellas T, Argacha P, Acosta J, et al. Estimation of tumor size in breast cancer comparing clinical examination, mammography, ultrasound and MRI-correlation with the pathological analysis of the surgical specimen. *Gland Surg*. 2017;6:330-335.