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BRIEF REPORT

The effect of hormone therapy on breast density following risk-reducing salpingo-oophorectomy in women with an increased risk for breast and ovarian cancer

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Abstract

Objective: To compare the effect of tibolone to conjugated estrogens with medroxyprogesterone-acetate (CEE + MPA) on breast density, as a predictor for breast cancer risk, in women with a high risk of breast and ovarian cancer.

Methods: Women aged 30-50 ($N = 114$) who had undergone risk-reducing salpingo-oophorectomy (RRSO) were randomized to tibolone or CEE + MPA.

Results: Breast density decreased 46% after RRSO in untreated women, 39% after treatment with tibolone, and 17% after treatment with CEE + MPA; the decrease in breast density after CEE + MPA was significantly different compared with that of untreated women ($P = 0.017$).

Conclusions: A decline in breast density is seen after premenopausal RRSO despite the use of both CEE + MPA or tibolone, although lower breast density is seen after tibolone use.

Key Words: BRCA1 – BRCA2 – Breast density – Hormone therapy – Risk-reducing salpingo-oophorectomy – Tibolone.

H ormone therapy (HT) in women with premature menopause after risk-reducing salpingo-oophorectomy (RRSO) aims to alleviate postmenopausal symptoms. Interestingly, HT may also improve long-term health, as a meta-analysis by Muka et al[1] showed that even long-term overall mortality may be increased in women with

premature menopause (such as those undergoing RRSO). However, there are concerns about an increased breast cancer risk, particularly in women with an existing high breast cancer risk due to a genetic predisposition such as a *BRCA1/2* mutation.[2,3] Alternatives such as tibolone are therefore under investigation. In vitro, tibolone stimulates apoptosis in glandular mammary tissue, reduces proliferation, and blocks local estradiol activity.[4]

The current study was inspired by reports that breast density increases in postmenopausal women after estrogen plus progestin but not after tibolone.[5,6] Mammographic breast density is a predictor for breast cancer risk.[7-12] Our goal was to investigate the effects of HT on breast density after RRSO in women with a genetic predisposition for ovarian and/or breast cancer.

METHODS

From 2004 to 2013, women were included based on a familial predisposition for breast and ovarian cancer, including a proven *BRCA1/2* mutation or if at 50% risk of being a carrier (as member of a *BRCA1/2* family), age 30 to 50 years, and an RRSO within the past 5 years or a planned RRSO within 6 months of randomization. Primary reasons for exclusion were HT or oral contraceptive use in the 3 months before

randomization, a history of breast cancer or prophylactic mastectomy, or any contraindication to HT.

In this national open label study, after giving informed consent, women were randomized 1:1 to receive either 0.625 mg conjugated equine estrogens and 5 mg medroxyprogesterone acetate continuously combined (CEE + MPA), or 2.5 mg tibolone continuously (Fig. 1). Currently, conjugated estrogens are not commonly prescribed in clinical practice. The comparison group consisted of women eligible for the study but who declined HT after RRSO. Treatment and follow-up, including mammography,

CONSORT

TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

Enrollment

Assessed for eligibility (n=170)

Excluded (n= 56)
- ♦ Not meeting inclusion criteria (n= n/a)
- ♦ Declined to participate (n=56)
- ♦ Other reasons (n= n/a)

Randomized (n=114)

Allocation

Allocated to tibolone (n=58)
- ♦ Received allocated intervention (n=58)
- ♦ Did not receive allocated intervention (n=0)

Allocated to CEE+MPA (n=56)
- ♦ Received allocated intervention (n=55)
- ♦ Did not receive allocated intervention (reason not given/participant refused) (n=1)

Follow-Up

Lost to follow-up (n=0)

Lost to follow-up (n=0)

Analysis

- ♦ Excluded from analysis (missing baseline mammogram, RRSO >2 months before enrollment) (n=27)

Analyzed ITT (n=31)
- - Discontinued intervention (SAE after 1 week) (n=1)

- ♦ Excluded from analysis (missing baseline mammogram, RRSO >2 months before enrollment) (n=21)
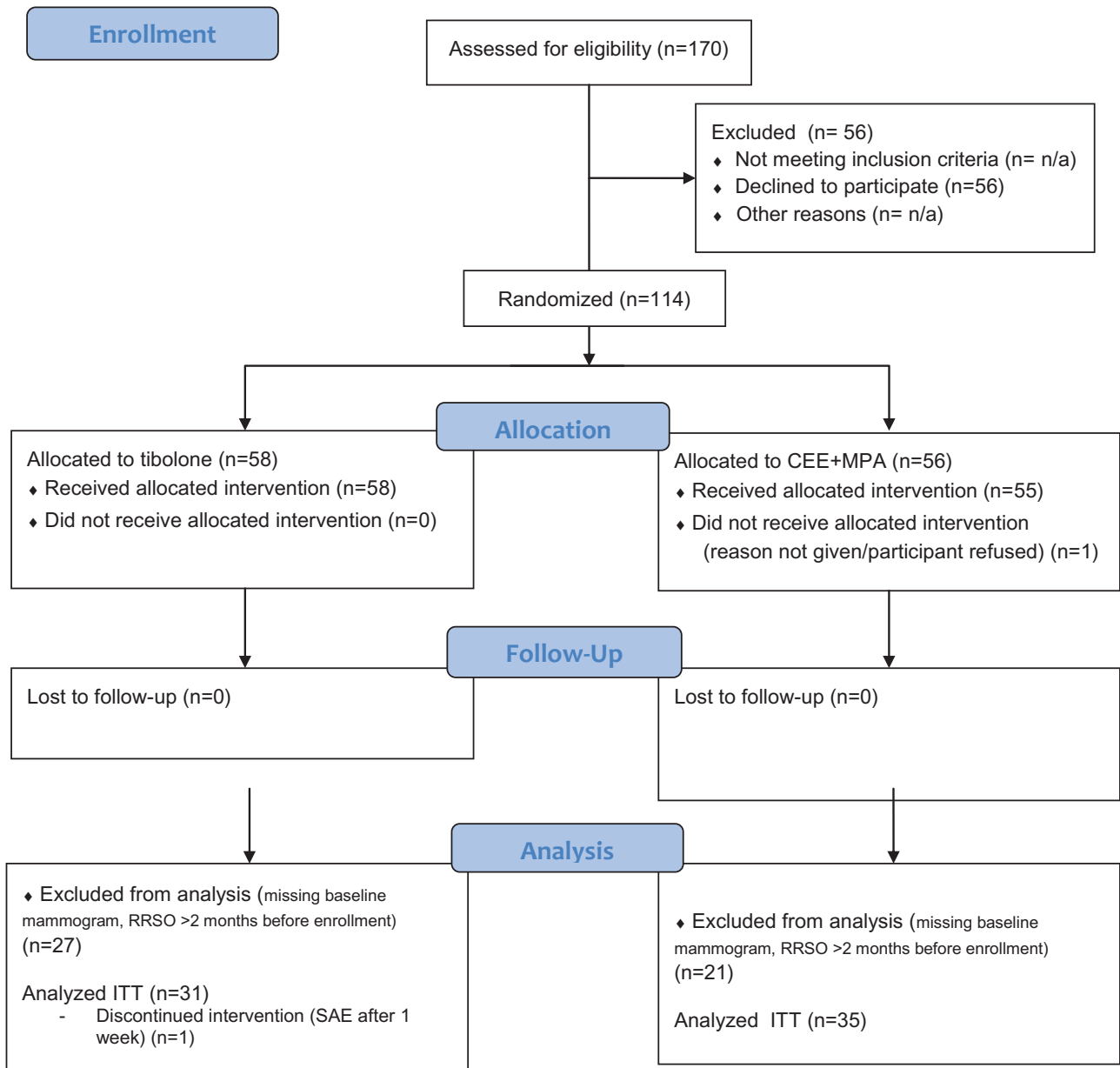
Analyzed ITT (n=35)

FIG. 1. Consort flow diagram. CEE + MPA, conjugated equine estrogens + medroxyprogesterone acetate; ITT, intention-to-treat; RRSO, risk-reducing salpingo-oophorectomy; SAE, serious adverse event.

TABLE 1. *Baseline characteristics of study participants*

Trial arm	CEE + MPA $N=35$	Tibolone $N=31$	Comparison group $N=24$	P value[a]
Age at baseline mammogram, median (range)	39.8 (34.0-45.3)	40.9 (36.8-48.4)	44.3 (35.3-48.8)	<0.001 0.034[b] <0.001[c] 0.244[d]
Age at RRSO, median (range)	40.6 (34.8-45.6)	41.2 (37.6-49.0)	44.7 (35.7-49.4)	<0.001 0.025[b] <0.001[c] 0.299[d]
Baseline breast density (BI-RADS)	*N* (%)	*N* (%)	*N* (%)	0.610
A	4 (11)	2 (6)	4 (17)	0.508[b]
B	14 (40)	9 (29)	9 (38)	0.634[c]
C	15 (43)	19 (61)	11 (46)	0.398[d]
D	2 (6)	1 (3)	0	
Time in days between RRSO and baseline mammogram, median (range)[e]	−117 (−361 to 46)	−139 (−308 to 18)	−114.5 (−301 to 41)	0.415

CEE + MPA, conjugated equine estrogens + medroxyprogesterone acetate; RRSO, risk-reducing salpingo-oophorectomy.
[a]The following statistical tests were used: One-way ANOVA for age at baseline mammogram and RRSO; Chi-squared test for baseline breast density; Kruskal-Wallis test for time between RRSO and baseline mammogram.
Individual comparisons: [b]CEE + MPA vs tibolone; [c]CEE + MPA vs comparison group; [d]tibolone vs comparison group.
[e]Negative value indicates that the mammography took place before RRSO.
Breast density (BI-RADS) A. The breasts are almost entirely fatty; B. There are scattered areas of fibroglandular density; C. The breasts are heterogeneously dense; D. The breasts are extremely dense.

were planned for at least 2 years after inclusion, with 2015 as the last year of follow-up.

The effect size for the power analysis was derived from Lundstrom et al[6] who found higher breast densities in 46% to 50% of women receiving estradiol + norethisterone acetate, compared with 2% to 6% for tibolone. Fifty-five women per arm were determined to be required to detect an 18% difference in the proportion of women in each group with at least one BI-RADS/ACR density category increase, at 80% power and an alpha of 0.05, using a chi-squared test or Fisher exact test for proportions. All mammograms were re-evaluated by an independent radiologist (in 2014), blinded for treatment arm, according to the BI-RADS 5th edition density score.[13] The analyses were based on revised scores and the intention-to-treat principle, with the primary endpoint being change in BI-RADS classification category after 2 years of follow-up in the two trial arms as well as the observation arm. Proportions with a change in breast density were compared between groups, using the Chi-squared or Fisher exact test for proportions. A paired differences test was performed to compare baseline and endpoint breast density. To account for the effect of differences at baseline, a logistic regression model was fitted for the outcome variable change in breast density, yes versus no. The model was adjusted for age and breast density at baseline. All analyses were performed using Stata, releases 15 and 16 (StataCorp LLC, TX).

RESULTS

Fifty-six women were randomized to CEE + MPA and 58 women to tibolone. An additional 56 women were included in the comparison group. To improve comparability, only women who underwent RRSO no more than 2 months prior to randomization were included in the final analysis. To ensure a proper baseline density measurement, a further requirement was the availability of a mammogram taken less than 1 year before randomization. Under these more stringent criteria, 35, 31, and 24 women were included in the CEE + MPA, tibolone, and the comparison groups, respectively. Thirty women were carriers of a *BRCA2* mutation, 44 of a *BRCA1* mutation, and 16 were considered at high risk for hereditary breast or ovarian cancer. Median ages at the time of baseline mammogram and at the time of RRSO differed between the three groups (Table 1). There was no difference in baseline breast density ($P = 0.610$), or in time interval between RRSO and baseline mammogram ($P = 0.415$).

As shown in Table 2, according to the BI-RADS classification no participant had an increase in breast density category after 2 years of follow-up. Among those receiving CEE + MPA, breast density was reduced by one density category in six women (17%) and unchanged in 29. In the tibolone group, one woman showed a two-category reduction (3%), 11 women (35%) a one-category reduction, and 19 showed no changes to breast density. Comparing the HT treatment arms, the proportion of women with decreased breast density was 39% (12/31) for tibolone, but only 17% (6/35) for CEE + MPA ($P = 0.05$, or 0.059 with Fisher exact test). Furthermore, 46% of women in the comparison group also showed a decrease of one BI-RADS density category, comparable to the tibolone arm ($P = 0.595$) but significantly more than in the CEE + MPA arm ($P = 0.017$). The test for paired differences showed that in all groups, overall group breast density at the endpoint was significantly decreased compared with baseline. Table 3 shows the logistic regression model, assessing modification of change in breast density by age and baseline breast density. Adjusting for age and baseline breast density, odds of decreased breast density were significantly higher in the tibolone and comparison groups, when compared with the CEE + MPA group (odds ratio 3.99, $P = 0.034$ and odds ratio 11.2, $P = 0.002$ for the tibolone and comparison groups respectively).

TABLE 2. *Comparison of changes in categorical breast density measurements*

Trial arm	CEE + MPA N = 35 (%)	Tibolone N = 31 (%)	Comparison group N = 24 (%)	Chi-squared test for proportions[a]
Change in (BI-RADS categories) breast density over 2 years				0.05[b,e], 0.017[c], 0.595[d]
0	29 (83)	19 (61)	13 (54)	
−1	6 (17)	11 (36)	11 (46)	
−2	0	1 (3)	0	
Test for paired differences.[f]	P = 0.031	P < 0.001	P = 0.001	

CEE + MPA, conjugated equine estrogens + medroxyprogesterone acetate.
[a]The −1 and −2 categories are combined for the chi-squared tests, and therefore compare the proportions with a decrease in breast density.
[b]CEE + MPA vs tibolone.
[c]CEE + MPA vs comparison group.
[d]Tibolone vs comparison group.
Negative change represents a decrease in BI-RADS category, eg, B to A (−1), or D to B (−2).
[e]Fisher exact test for the proportion with a change in breast density between CEE + MPA and tibolone, P = 0.059 (two-sided).
[f]Wilcoxon signed-rank test was used, which is the nonparametric variant of the paired t test, suitable for the ordinal density variables. In all groups, the endpoint density was significantly different from baseline.

DISCUSSION

Two years after premenopausal RRSO, only 17% of women treated with CEE + MPA showed a decrease in breast density, a substantial difference compared with tibolone (39%) and a significant difference to untreated women (46%). When started soon after RRSO, CEE + MPA seems to counteract the natural decline in breast density that occurs after RRSO, which did not result in an increase above the premenopausal baseline. It should be noted that the BI-RADS classification is rather crude compared with automated percentage-density measurements and may not detect mild or moderate changes in density. This likely explains why not all untreated women experienced a decline in density. Already-postmenopausal women showed the opposite effect, usually increasing breast density once started on HT.[5,6,14]

In this study, all women willing to take hormones were randomized, as would be done in any randomized controlled trial. The exception here is that our "comparison group" is not a true control group. Instead, the CEE + MPA group (the standard HT at the time) functions as the control group in the randomized experiment. The comparison with the non-hormone-taking comparison is not part of the randomized design, but included to provide as much information as possible.

Consequently, the women who declined taking hormones are as a group therefore likely to be different from the randomized groups.

It should be noted that recruitment for this study was difficult, as unfortunately, a high percentage of potentially eligible women had either been treated for breast cancer or had a risk-reducing mastectomy. Furthermore, at the time of the study, the patient advocacy committee in the Netherlands recommended against HT, as it supported the view that RRSO without HT would reduce breast cancer risk, while RRSO with HT would increase breast cancer risk. This further reduced the willingness to accept HT. In addition, women were reluctant to be randomized, preferring tibolone or CEE + MPA (or simply no HT at all). Furthermore, we felt it necessary to exclude women in whom RRSO was performed too long ago (as this may change treatment effects), or in whom a proper baseline density measurement was missing. The result was that 35, 31, and 24 women for the CEE + MPA, tibolone, and comparison groups were eligible for analysis, instead of the calculated group size of 55. A postrandomization selection like this may affect baseline characteristics, which are normally assumed to be equally distributed due to randomization, and may necessitate adjustment for baseline

TABLE 3. *Logistic regression model to assess modification of changes in breast density by baseline differences*

	Odds ratio	Standard error	P value
Group			
CEE + MPA	Reference	n/a	n/a
Tibolone	3.99	2.61	0.034
Comparison group	11.2	8.98	0.002
Baseline breast density			
A	n/a	No decrease possible	
B	0.09	0.13	0.091
C	0.13	0.19	0.146
D	n/a	Collinearity[a]	
Age at time of participation (per year increase)	0.89	0.08	0.199
Constant	136.7	517.3	0.194

The outcome variable of the logistic regression model is binary, ie, decrease in breast density, yes or no.
CEE + MPA, conjugated equine estrogens + medroxyprogesterone acetate.
[a]In the tibolone group, the only participant with a breast density category score of D had a decrease in breast density, leading to exact correlation of change in breast density and baseline breast density.

differences in analyses. Despite this, differences in age at RRSO and at baseline mammogram were small and the natural decline in breast density with age is in any case limited (0.2%-1% per year).[15-17] Furthermore, no differences were noted in time between RRSO and baseline mammogram or in baseline breast density. The differences in breast density that arose during follow-up are therefore unlikely to be confounded by the above-mentioned baseline factors. This was also demonstrated by the adjusted logistic regression model.

Our initial assumption, based on aforementioned studies, was that both HT regimens would increase breast density when begun immediately after surgical menopause, but with a larger effect for CEE + MPA. However, the current study suggests that women who undergo premenopausal RRSO predominantly show a decrease in breast density, a decrease more potently counteracted by CEE + MPA than tibolone. The most likely explanation is that loss of endogenous hormone production after RRSO leads to loss of breast density, which is not completely compensated by hormone therapy. Considering breast density as a predictor for breast cancer, our results variously suggest that 1) the influence of tibolone on breast density and possibly breast cancer risk after RRSO may be comparable to no HT, and 2) CEE + MPA enhances breast density and possibly breast cancer risk after RRSO compared to the comparison group, and possibly tibolone.

It is important to note that risk-reducing policies may change over time. The acceptance of hysterectomy (ie, performed with concomitant RRSO) varies around the world and women may fear (both short- and long-term) side effects of removing the uterus. Therefore, the effect of different hormonal strategies on breast density, and by extension breast cancer risk, is important to study further in RRSO recipients. Promising strategies for women with an intact uterus include newer therapies such as the combination of bazedoxifene with conjugated estrogens (which does not seem to cause endometrial hyperplasia or increased breast density,[18,19] or more familiar combinations, such as estrogens combined with a progestogen-releasing intrauterine device (ie, Mirena)). The long-term effects of these strategies on breast cancer risk are not certain, and still require further study.

CONCLUSION

Our results suggest that tibolone has the least effect on the natural decrease in breast density after premenopausal RRSO, similar to no HT in this regard. Whether this actually equates to a reduced breast cancer risk remains to be investigated, as studies concerning our target population are scarce and results inconclusive. A recent meta-analysis of HT in postmenopausal women reported an increased breast cancer risk for women using tibolone (relative risk of 1.57, 95% CI: 1.43-1.72, during 5-14 y of use).[20] Somewhat contradictory, a Cochrane review of randomized controlled trials of tibolone use in postmenopausal women concludes that breast cancer risk may not be increased (odds ratio 0.52, 95% CI: 0.21-1.25), although trial

quality was very low, according to their assessment. Breast cancer recurrence risk, however, was shown to be increased by tibolone in two moderate quality trials (odds ratio 1.5, 95% CI: 1.21-1.85).[21] Of note, is that most of these results pertain to women quite a bit older than our study population. The mean age was around 52 to 55 in the Cochrane review and 50 in the meta-analysis (although not reported for the tibolone-subgroup specifically), and the results may therefore not fully apply for women in our study, who are undergoing RRSO at a young age (ie, around the age of 40). Results may also differ when breast cancer risk from HT in young postmenopausal women is compared with risk in premenopausal women of the same age. Nonetheless, potential risks and benefits will need be considered when prescribing or investigating HT, such as tibolone, in young postmenopausal women.

REFERENCES

1. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: a systematic review and meta-analysis. *JAMA Cardiol* 2016;1:767-776.
2. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321-333.
3. Beral V; Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003; 362:419-427.
4. Gompel A, Chaouat M, Jacob D, Perrot JY, Kloosterboer HJ, Rostene W. In vitro studies of tibolone in breast cells. *Fertil Steril* 2002;78:351-359.
5. Valdivia I, Campodonico I, Tapia A, Capetillo M, Espinoza A, Lavin P. Effects of tibolone and continuous combined hormone therapy on mammographic breast density and breast histochemical markers in postmenopausal women. *Fertil Steril* 2004;81:617-623.
6. Lundstrom E, Christow A, Kersemaekers W, et al. Effects of tibolone and continuous combined hormone replacement therapy on mammographic breast density. *Am J Obstet Gynecol* 2002;186:717-722.
7. Fabian CJ, Kimler BF. Mammographic density: use in risk assessment and as a biomarker in prevention trials. *J Nutr* 2006;136:2705S-2708S.
8. Fabian CJ, Kimler BF, Brady DA, et al. A phase II breast cancer chemoprevention trial of oral alpha-difluoromethylornithine: breast tissue, imaging, and serum and urine biomarkers. *Clin Cancer Res* 2002; 8:3105-3117.
9. Decensi A, Costa A. Recent advances in cancer chemoprevention, with emphasis on breast and colorectal cancer. *Eur J Cancer* 2000;36:694-709.
10. Knight JA, Martin LJ, Greenberg CV, et al. Macronutrient intake and change in mammographic density at menopause: results from a randomized trial. *Cancer Epidemiol Biomarkers Prev* 1999;8:123-128.
11. Byrne C, Ursin G, Martin CF, et al. Mammographic density change with estrogen and progestin therapy and breast cancer risk. *J Natl Cancer Inst* 2017;109:djx001.

12. Boyd NF, Lockwood GA, Martin LJ, et al. Mammographic densities and risk of breast cancer among subjects with a family history of this disease. *J Natl Cancer Inst* 1999;91:1404-1408.

13. American College of Radiology, D'Orsi CJ. ACR BI-RADS Atlas: Breast Imaging Reporting and Data System; Mammography, Ultrasound, Magnetic Resonance Imaging, Follow-up and Outcome Monitoring, Data Dictionary. Reston, VA: American College of Radiology; 2013. Available at: https://radiologyassistant.nl/breast/bi-rads/bi-rads-for-mammography-and-ultrasound-2013. Accessed July 16, 2021.

14. Azam S, Jacobsen KK, Aro AR, Lynge E, Andersen ZJ. Hormone replacement therapy and mammographic density: a systematic literature review. *Breast Cancer Res Treat* 2020;182:555-579.

15. Lokate M, Stellato RK, Veldhuis WB, Peeters PH, van Gils CH. Age-related changes in mammographic density and breast cancer risk. *Am J Epidemiol* 2013;178:101-109.

16. Stone J, Warren RM, Pinney E, Warwick J, Cuzick J. Determinants of percentage and area measures of mammographic density. *Am J Epidemiol* 2009;170:1571-1578.

17. Li T, Sun L, Miller N, et al. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14: 343-349.

18. Pinkerton JV, Harvey JA, Pan K, et al. Breast effects of bazedoxifene-conjugated estrogens: a randomized controlled trial. *Obstetr Gynecol* 2013;121:959-968.

19. Pickar JH, Yeh IT, Bachmann G, Speroff L. Endometrial effects of a tissue selective estrogen complex containing bazedoxifene/conjugated estrogens as a menopausal therapy. *Fertil Steril* 2009;92: 1018-1024.

20. Collaborative Group on Hormonal Factors in Breast C. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. *Lancet* 2019;394:1159-1168.

21. Formoso G, Perrone E, Maltoni S, et al. Short-term and long-term effects of tibolone in postmenopausal women. *Cochrane Database Syst Rev* 2016;10:CD008536.