Reduction Mammographic Density with Use of a Gonadotropin-Releasing Hormone Agonist–Based Chemoprevention Regimen in BRCA1 Carriers

Jeffrey N. Weitzel,1,2 Saundra S. Buys,4 William H. Sherman,5 Anna Marie Daniels,6 Giske Ursin,2,7 John R. Daniels,3,6 Deborah J. MacDonald,1 Kathleen R. Blazer,1 Malcolm C. Pike,2,6 and Darcy V. Spicer3,6

Abstract

Purpose: Women with a BRCA mutation (BRCA1mut) need risk reduction options beyond mastectomy and oophorectomy. We evaluated the efficacy, safety, and tolerability of hormonal chemoprevention with a gonadotropin-releasing hormone agonist (GnRHA) with low-dose add-back steroids in BRCA1mut carriers.

Experimental Design: The 12-month open label clinical trial used the GnRHA deslorelin, ultralow-dose estradiol (E2), and replacement testosterone, administered via daily intranasal spray in premenopausal women with a BRCA1mut, and intermittent oral medroxyprogesterone acetate. The end points included mammographic percent density, bone mineral density, endometrial hyperplasia, symptom inventory, and quality of life (Medical Outcomes SF-36 survey).

Results: Six of eight BRCA1mut women (mean age, 30.3 years; range, 25–36 years) completed the study. Mammographic percent density was significantly reduced at 12 months (median absolute mammographic percent density decrease, 8.3%; P = 0.043), representing a 29.2% median reduction in mammographic percent density. Bone mineral density remained within reference limits for all participants; there were no cases of atypical endometrial hyperplasia and menses resumed within a median of 67 days (range, 35–110 days) after last drug treatment day. The treatment was well tolerated; hypoestrogenic side effects were minimal and transient; and there were no significant changes in quality of life.

Conclusions: The GnRHA deslorelin, with low-dose add-back steroids, was well tolerated and significantly decreased mammographic percent density in BRCA1mut carriers. This regimen may reduce breast cancer risk and improve the usefulness of mammographic surveillance by reducing density. This is the first demonstration, to our knowledge, of a direct reduction of mammographic densities in young BRCA1mut carriers.

There is overwhelming evidence for a major role of ovarian hormones in the etiology of sporadic breast cancer. Premenopausal oophorectomy significantly reduces breast cancer risk in BRCA mutation (BRCA1mut) carriers, implicating ovarian hormones in hereditary breast cancer as well (1, 2).

Because of the extraordinary risk for both breast and ovarian cancer, mastectomy is one current risk reduction option for BRCA1mut carriers, and salpingo-oophorectomy is recommended after completion of childbearing (1–5). A number of concerns limit the appeal of available strategies of hormonal breast cancer risk reduction to young women. In particular, oophorectomy is an irreversible procedure and eliminates fertility, and concerns about safety and efficacy limit the acceptability of selective estrogen receptor modifiers in such women (6).

A lesser reduction in the levels of premenopausal ovarian hormones than is achieved with oophorectomy has the potential to decrease breast cancer risk (7–9). Such a reduction can be achieved with the use of a gonadotropin-releasing hormone agonist (GnRHA) with add-back low-dose sex steroids. The use of a GnRHA alone is associated with menopausal vasomotor symptoms in the short term and osteoporosis and...
vaginal atrophy in the long term. We have previously proposed that these symptoms could be mitigated by low-dose add-back steroids. A prototype GnRHA hormonal chemoprevention regimen, which used 7.5-mg leuprolide acetate depot by intramuscular injection every 28 days and oral administration of conjugated equine estrogen and intermittent progestins in women who were at increased risk of breast cancer based on empirical risk models, showed decreased mammographic densities compared with placebo and excellent patient acceptability (10, 11).

Mammographic densities are strongly positively related to breast cancer risk (12–16). Limited sensitivity of mammography in premenopausal women, predominantly due to the increased mammographic density in young women, has been observed in sporadic cancers and in BRCA1 carriers (17, 18).

Changes in mammographic density are considered a possible marker of hormonal modification of breast cancer risk (19–21). Oophorectomy is associated with a reduction in mammographic densities, as is tamoxifen use, particularly in younger women (22). The purpose of the pilot study reported here was to examine the effect on mammographic density, and the safety and tolerability of a 12-month regimen of an intranasally administered drug combination product consisting of the GnRHA deslorelin, 17β-estradiol (E2), and testosterone in premenopausal BRCA1 carriers. The dose of add-back E2 was designed to achieve a serum level of E2 60% of the lowest level seen at any time in the normal menstrual cycle while obviating hypoestrogenic symptoms (11). The dose of add-back testosterone was designed to replace the ovarian testosterone lost through the action of the GnRHA. Intermittent (every 13 weeks) oral medroxyprogesterone acetate was administered to protect the endometrium (11).

The known hypoestrogenic effects of GnRHA treatment are loss of bone mineral density and hot flashes. Hot flashes, as well as quality of life, were monitored throughout the study. Bone mineral density measurements and endometrial biopsies were obtained at 12 months to monitor for bone loss and endometrial hyperplasia.

Materials and Methods

Design. The trial was a single-arm, open-label study of 12-month treatment duration. Study participants were recruited from high-risk clinical populations at the City of Hope Cancer Center in California, Huntsman Cancer Institute at the University of Utah, and the Columbia Presbyterian Medical Center in New York. The study was approved by the respective institutional review boards.

Eligible participants had a documented deleterious BRCA1 mutation. At baseline, all participants were (a) premenopausal, between ages of 21 and 42 years; (b) in generally good health (including normal blood count, adequate hepatic and renal function, and baseline lumbar spine bone mineral density within reference range as assessed by quantitative digital radiography); (c) had no evidence of breast cancer, were nonsmokers, and had a prior tubal ligation or were willing to use a nonhormonal barrier method of contraception; and (d) provided signed informed consent.

Subjects were ineligible if they (a) had received a GnRHA treatment within 12 months of study entry or were currently using hormones, corticosteroids, or other specified medications; (b) had nasal polyposis, rhinitis, or sinusitis requiring current treatment or treatment for more than 3 months in the previous year; (c) were pregnant or breast-feeding within 12 months of study entry; (d) had hyperthyroidism; (e) had a history of osteoporosis or multiple fractures; or (f) had any contraindication to the use of estrogens or known allergy to any of the compounds being administered.

Study regimen (IND# 48,397). Throughout the study period, each subject self-administered a 100-μl metered-dose nasal spray containing the GnRHA deslorelin acetate (1 mg) and a 100-μl metered-dose nasal spray containing E2 (0.30 mg) and testosterone (0.275 mg) each morning (Balance Pharmaceuticals, Inc., Santa Monica, CA). Individual vials contained a 1-month supply of drug. Used vials were returned to the study site and each month and deslorelin levels were assessed on days 29, 169, and 337 and indicated compliance. Subjects took 10-mg oral medroxyprogesterone acetate for 14 days every 13 weeks.

End points. Bilateral mammograms (cranio-caudal and mediolateral views) were obtained at baseline and after 6 and 12 months of study medications. Change in mammographic density was quantitated by an established method (23). Cranio-caudal mammograms were digitized at a resolution of 150 pixels/in. using a CobarScan CX812T scanner (Radiographic Digital Imaging, Torrance, CA). A computer program was used to assign a pixel value of 0 to the darkest (black) shade in the image and a value of 255 to the lightest (white) shade with shades of gray assigned intermediate values (23). The total area of the breast was outlined using a computerized outlining tool and the number of pixels within that outline gives the “total breast area.” Subsequently, the reader outlined a region of interest that contained the entire breast but excluded white artifacts such as the pectoralis muscle, prominent veins, and singular fibrous strands. A tinting tool was used to apply a yellow tint to pixels with gray levels at or above some threshold X within the region of interest. The reader searched for the best threshold where all pixels ≤X within the region of interest were considered to represent mammographic densities. The numbers of tinted pixels within the region of interest represent the area of absolute mammographic density. Mammographic percent density, or the fraction (%) of the breast with densities, was the ratio of absolute mammographic density to the total breast area. Mammograms from each patient were read in a random order. A single reader (G.U.) reviewed all mammograms for the study and was blinded to the time points of the mammograms.

The primary efficacy analysis is based on the comparison of mammographic densities at baseline and 12 months. In all comparisons, both right and left breast mammograms were used. The individual subjects’ mammograms were paired (same breast at baseline versus 12 months) to assess mammographic changes. The average change for both breasts was calculated for each subject and used in the statistical analysis. Wilcoxon non-parametric rank-sum test was used to assess statistical significance. The 6-month mammograms were used in a similar manner to study whether mammographic density changes at 12 months could have been detected as early as 6 months.

Bone mineral density of the lumbar spine was determined at the screening visit and at 6 and 12 months. Each measurement was done in duplicate, separated by the subject getting off and back on to the measurement table.

Normal endometrial biopsy was required for study entry. Possibility of endometrial hyperplasia was monitored by endometrial biopsy at 12 months.

Health-related quality of life was assessed at baseline, 6 months, and at end of study using the instruments used in the Breast Cancer Prevention Trial (24), which include the Medical Outcomes SF-36 (25) and a validated 54-item inventory describing commonly reported physical and psychological symptoms, as well as vasomotor symptoms that have been associated with menopause (e.g., hot flashes, joint pains, forgetfulness, difficulty concentrating, and vaginal dryness; ref. 26). Some new items were added to monitor for specific symptoms/toxicities from the study medication (e.g., nasal irritation, unpleasant taste after drug spray).

After completing study treatment, subjects were queried by phone at regular intervals until their return of menstrual cycles.

Serum measurements. Serum E2, testosterone, and progesterone were measured on treatment day 1 before drug administration. E2 and
testosterone levels were measured again 20 min after first drug dose. Hormone levels were repeated on days 29, 169, and 337.

**Results**

Eight $BRCA1^{mut}$ carriers signed consent forms, were found eligible on screening evaluations, and initiated drug treatment. The mean age was 30.3 years and ranged from 25 to 36 years; six were non-Hispanic Caucasian and two were Hispanic. Mean body mass index was 30.5 [without one outlier (body mass index, 57), the mean body mass index for the remaining seven is 26.7]. One participant discontinued study on day 93, citing psychosocial stressors unrelated to study. Another participant moved out of the country after the midpoint evaluation. Six completed the 12-month study per protocol.

Estrogen levels at 3, 6, and 12 months were appropriately decreased with mean $E_2$ levels of 28 pg/mL (49% of baseline value) and mean testosterone levels of 24 ng/dL.

**Mammographic density**

One of the six $BRCA1^{mut}$ carriers who completed the study had very few mammographic densities at baseline, and, although even these disappeared at the 12-month exam, her results were excluded from the analysis of mammographic percent density results. Table 1 shows the mammographic density measurements for the remaining five $BRCA1^{mut}$ carriers at baseline and 12 months; mammographic percent density was significantly reduced at 12 months, with a median absolute decrease of 8.3% (two-sided $P = 0.043$); this represented a 29.2% median reduction in mammographic percent density. The reduction at 6 months was approximately half this amount and was not statistically significant. Although one cannot distinguish between the 0.8% and 0.0% mammographic percent density results, inclusion of the sixth subject results in a median absolute decrease of 7.0% (rather than 8.3%) at 12 months across all participants and the median percent change at 12 months is 12.9%, whereas the two-sided $P$ value changes from 0.043 to 0.028, becoming slightly more statistically significant. Figure 1 shows an example of the change in appearance of a mammogram, with an obvious reduction in densities after 12 months on study treatment.

**Safety**

*Endometrial hyperplasia.* None of the $BRCA1^{mut}$ carriers had evidence of hyperplasia at the 12-month biopsy.

*Bone mineral density.* Five of the six $BRCA1^{mut}$ carriers had a bone mineral density measurement at 12 months. Their average bone mineral density at baseline was 1.008; at 12 months, this had increased to 1.011. Bone mineral density measurements remained within reference limits for all subjects.

*Quality of life.* Complete quality of life, menopausal symptoms, and sexual functioning questionnaires were obtained on five of the six $BRCA1^{mut}$ carriers. No significant changes were noted in the average scores over the 12-month period of the study. For seven of the eight scales, one half or more of the subjects reported no change or improvement.

*Return of menses.* The median time to return of menses was 66.7 days (range, 35-110 days).

No serious adverse events were reported during the course of the study. Adverse events were mild in severity and generally unrelated to treatment. Those related to treatment were most commonly hypoestrogenic side effects that resolved without intervention or following a prescribed 50% increase in replacement spray (one subject). There was no significant local irritation from administration of the nasal spray.

**Discussion**

The results of this study indicate that a self-administered GnRHA-based regimen with low-dose add-back steroids can significantly reduce the mammographic densities of $BRCA1^{mut}$ carriers with a known high risk of breast cancer. Reduction in densities was evident after 6 months of treatment but to only half the extent seen at 12 months. This is the first demonstration, to our knowledge, of direct modulation of breast density in $BRCA1^{mut}$ carriers. We recruited only a single $BRCA2$ carrier to the study so that we are unable to make any recommendations about such women on the basis of actual results with such a regimen.

All mammograms from the 12-month time point were obtained while the women were still on the treatment, but we did not require the baseline mammograms to be obtained in a certain phase of the cycle, and we had to assume that they could have been obtained in either the luteal or follicular phase. However, although we have previously described a

### Table 1. Mammographic percent density at baseline and at 12 mos in $BRCA1^{mut}$ carriers treated with a GnRHA-based regimen

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Baseline</th>
<th>12 mo</th>
<th>Change at 12 mo</th>
<th>% Change at 12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>COH-001</td>
<td>34.2</td>
<td>24.2</td>
<td>-10.0</td>
<td>-29.2</td>
</tr>
<tr>
<td>COH-002</td>
<td>53.2</td>
<td>44.9</td>
<td>-8.3</td>
<td>-15.6</td>
</tr>
<tr>
<td>COH-013</td>
<td>5.7</td>
<td>0.0</td>
<td>-5.7</td>
<td>-100.0</td>
</tr>
<tr>
<td>UTA-001</td>
<td>14.6</td>
<td>10.3</td>
<td>-4.3</td>
<td>-29.5</td>
</tr>
<tr>
<td>UTA-002</td>
<td>38.7</td>
<td>29.6</td>
<td>-9.1</td>
<td>-23.5</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td>-8.3</td>
<td>-29.2</td>
</tr>
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</table>
change in mammographic density during the menstrual cycle (27), this change is rather small (median increase of 1.4%). It is therefore unlikely that the median reduction of 8.3% observed in this study can be explained by variations across the menstrual cycle. In our previous study of high-risk women using monthly leuprolide acetate injections and oral estrogens, we found a reduction in mammographic densities very similar to the reduction found in this study (10, 23, 28).

There is considerable evidence that the reduction in mammographic densities reflects a reduction in breast epithelial cells and suggests a high likelihood that this regimen will reduce breast cancer risk in BRCA1 mutant carriers. Further, there is strong data, from observations of the effect of risk-reduction oophorectomy in BRCA1 mutant carriers, that a reduction in exposure to ovarian hormones will significantly reduce breast cancer risk. The protective effect of risk-reduction oophorectomy in BRCA1 mutant carriers was not significantly attenuated by hormone therapy analogous to the levels used in this trial (29). Consequently, it is highly likely that this regimen would be associated with a reduction in breast cancer risk. Although the ultimate intent of the regimen is prevention, the decrease in breast density will also likely lead to more effective mammographic screening in women. Recent data indicate that the sensitivity of mammography in young women at increased breast cancer risk is only ~36% (17, 18). Mammographic densities were cited as one of the factors limiting the efficacy of mammography. Although the sensitivity of breast magnetic resonance imaging was higher (~78%), given the considerable expense, high false-positive rate, and limited availability of high-quality magnetic resonance imaging services, an intervention that reduces mammographic density may be a cost-effective approach to improved breast cancer screening in high-risk women. A current phase II biomarker trial with the same regimen will explore the effect of the regimen on tissue changes associated with changes in breast density, as well as the effect on magnetic resonance images.

The participants in this study remained healthy by all measures and did not have any decrement in their quality of life. Bone density was unchanged over the course of the study. There were no adverse effects on the uteri of participants on our intermittent progestin regimen as there were no cases of atypical endometrial hyperplasia. There was a reduction in bone density was unchanged over the course of the study. Hypoestrogenic side effects were minimal. The lack of persistent vasomotor symptoms and maintenance of a good quality of life is likely a factor in the good compliance with the study regimen by participants and suggests the feasibility of longer-term use. The optimal duration of the intervention should be studied in future trials.

Thus, this study is a proof of principle that mammographic density and, presumably, breast cancer risk could be reduced in BRCA1 mutant carriers by an easily self-administered regimen of deslorelin and low-dose add-back steroids, sufficient to maintain a woman in good health, while preserving quality of life and reproductive options.

In addition, the regimen also may have potential as a contraceptive formula for young BRCA1 mutant carriers. It would presumably avoid any increase in breast cancer risk associated with oral contraceptive pills in BRCA1 mutant carriers (30, 31). We speculate that the induced cessation of ovulation may reduce ovarian cancer risk, analogous to the effect of oral contraceptive pills. In any event, this regimen clearly preserves options for surgical risk reduction. Current standard of care would still be to offer risk-reduction oophorectomy on completion of childbearing (5). Theoretically, a woman would continue to accrue risk reduction benefit from continued use of the regimen, but the optimal duration is unknown.

Challenges to accrual in this study for young well women included aversions to some procedures (e.g., endometrial biopsy), time out of work, travel and distance barriers, and concerns about exogenous steroids given the Food and Drug Administration and Institutional Review Board required listing of potential side effects, notwithstanding the fact that levels on study were less than physiologic norms for premenopausal women.

Although clearly speculative, given the small sample size of the current study, one could envision a long-term medicinal approach to breast and ovarian risk reduction in BRCA1 mutant carriers, with the GnRHA regimen as the cornerstone of early premenopausal years, breaks for childbearing, and perhaps transition to risk-reduction oophorectomy and selective estrogen receptor modifiers or aromatase inhibitors on completion of childbearing.

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