A Pilot Surrogate End Point Biomarker Trial of Perillyl Alcohol in Breast Neoplasia

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ABSTRACT

Purpose: Efficient strategies to screen promising agents in early phase development are essential for rapid progress in breast cancer chemoprevention. We report our experience with the natural compound perillyl alcohol (POH) administered in a short-term surrogate end point biomarker (SEB) protocol, using the “window” between diagnostic and definitive surgery.

Experimental Design: Eligible patients included those with a diagnosis of atypical ductal hyperplasia, ductal carcinoma in situ, lobular carcinoma in situ, or invasive carcinoma (<3 cm in size) that required further surgery. Thirty-seven of 267 women screened were enrolled in the study (14%). Five women received single-dose POH (1.5 g/m²) 2 days before surgery, 16 received escalating doses of POH (1.2 g/m² to 4.8 g/m²/day) for 2 days before surgery, and 16 served as untreated controls. Exploratory SEB analysis [estrogen receptor, progesterone receptor, proliferation, apoptosis, M6P/insulin-like growth factor (IGF)-2R, IGF1, IGF2 and transforming growth factor β] was conducted before and after POH.

Results: Only a small portion of the population screened entered the study. Reasons for nonparticipation included protocol eligibility, conflict of timing of surgery, miscellaneous logistical reasons, or patient’s choice. POH administration was well tolerated and did not interfere with surgical management. The power to observe changes in candidate SEB was diminished by a 44% incidence of cases in which the index lesion was not present in the definitive surgical specimen.

Conclusions: Preoperative POH exposure was safe and suitable for a more definitive phase II SEB study. Further investigations must overcome logistical obstacles to accrual, and they must focus on approaches to maximize tissue collection and to incorporate genomic analysis of target lesions.

INTRODUCTION

Tamoxifen is an established chemopreventive agent for women at high risk for developing breast cancer (1, 2). However tamoxifen is only partially effective and does not suppress estrogen receptor (ER)-negative disease. New chemopreventive agents must therefore be identified. Only a few definitive phase III breast cancer prevention trials can be conducted because the low event rate requires a large sample size, years of follow-up, and therefore high cost. Developing “early phase” clinical trial methodologies for chemopreventive agents akin to the phase I and phase II studies for traditional cancer therapeutics is therefore an important research priority. Ideally these studies should confirm not only the potential for chemoprevention but also answer questions about optimal dose and schedule. Chemoprevention trials of tamoxifen and raloxifene were based on secondary observations of reductions in new breast cancers made during analyses of placebo-controlled studies addressing their use in early-stage breast cancer and osteopenia/osteoporosis, respectively (1, 3). A similar level of preliminary evidence is not likely to be available for most candidate agents for chemoprevention trials.

In response to these issues, several investigators have proposed or initiated studies that focus on candidate surrogate end point biomarker (SEB) of the target lesion pathophysiology rather than on long-term cancer prevention (4–6). In such phase II designs, the end points used for classical antineoplastic agents, such as reduction in clinical or radiological assessments of tumor size, are replaced with evidence for reversal of one or more elements of the neoplastic phenotype, such as abnormal proliferation, cell survival, or aberrant gene expression. SEB may be tissue-based or might include changes in breast imaging (e.g., changes in mammographic density) or changes in serum biomarkers that may be associated with increased risk of developing the disease.

SEB studies are well established in the setting of prema-
lignant diseases that do not require surgical intervention such as leucoplakia of the oral mucosa (7) or Barrett’s esophagus (8). Such studies are more difficult to conduct in premalignant lesions requiring immediate surgical management, such as those occurring in the breast, because the index lesion is removed in a timely manner and is not amenable to subsequent follow-up. However, the need for sequential procedures to diagnose and remove invasive or preinvasive lesions provides an opportunity for short-term SEB studies. Intervals of several days to several weeks between procedures provide a window within which a chemopreventive agent can be administered to evaluate the effects of the agent on SEB modulation.

We chose to investigate perillyl alcohol [4-(1-methylethene)-1-cyclohexene-1-methanol; POH], a natural compound with preclinical evidence for chemopreventive activity, for a pilot perioperative SEB study. POH is found naturally in the essential oils of plants and citrus fruits and has been shown to inhibit various stages of mammary tumorigenesis in animal studies (9–12). Most notably, POH inhibits the formation of mammary tumors and reduces the size of established tumors in the 7,12-dimethylbenz(a)anthracene-rat mammary cancer model and is about five times more potent than the closely related monoterpenes, limonene (13, 14). Careful evaluation of the cellular basis of POH activity suggests that antitumor effect might occur through apoptosis, cell cycle arrest, and differentiation (15). The exact mechanism for the activity of this class of agents is unknown because the primary cellular effector(s) for POH have not been identified clearly. Exposure to POH results in up-regulation of the candidate tumor suppressor mannone 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) and increased signaling through the transforming growth factor β1 (TGFβ1) pathway (16–18). These data suggested that TGFβ1 and M6P/IGF2R might be useful candidate SEB for the action of POH against early breast lesions in human subjects.

In a rat mammary carcinoma model, treatment with POH for 1 to 48 hours was associated with change in apoptosis, and modulation of cell proliferation-related genes. TGFβ-related gene modulation was seen after 12 to 48 hours of POH exposure (19). POH has been examined in cancer patients for evidence of antineoplastic activity in phase I studies (20). POH was well tolerated with an maximum-tolerated dose of 2.4 g/m² three times per day. The most frequent side effects involved the gastrointestinal tract (such as nausea, vomiting, diarrhea, and gas-troesophageal reflux; refs. 20–22).

This report describes an investigation in which women with newly diagnosed malignant or premalignant breast lesions were asked to participate in a feasibility study of short-term exposure to POH immediately before surgery. Because the safety and tolerability of POH have not been established in the perioperative setting, and gastrointestinal symptoms were a particular concern for patients undergoing anesthesia, a dose escalation design was adopted to determine the maximum-tolerated dose for future studies.

Initially, we studied the safety of a single dose of POH with regard to blood cell counts, liver enzymes, wound healing, and anesthesia complications. We then used a dose escalation schema of a 48-hour treatment period to evaluate safety and to assess in a preliminary fashion the acute effects of the drug on candidate SEB. After establishing the safety of short-term POH, we hoped to evaluate the effects of a chronic POH administration on candidate SEB. The primary objectives of this study focused on feasibility and tolerability of POH administration. However, we also did an exploratory SEB analysis including generic measures of morphology, differentiation, apoptosis, and proliferation as well as several molecular biomarkers that are more specific to the effects of POH noted in animals, including M6P/IGF2R and TGFβ1 expression by immunohistochemistry.

**PATIENTS AND METHODS**

**Patients**

Between May 1999 and December 2000, patients were enrolled from the medical oncology, surgical oncology, and breast imaging clinics at Georgetown University Medical Center. The clinical trial received approval from the Georgetown University Medical Center Institutional Review Board, and all patients provided written informed consent. Women were eligible if they had a diagnostic (core, incisional, or excisional) breast biopsy containing atypical ductal hyperplasia, ductal carcinoma in situ (DCIS) with or without micro-invasion, lobular carcinoma in situ, or invasive carcinoma <3 cm (with or without DCIS). Positive surgical biopsy margins were required so that subsequent re-excision surgery was appropriate as a standard of care. Adequate hematologic, renal, and liver functions were required. Exclusion criteria included frequent vomiting or poor alimentation, concurrent acute or chronic medical or psychiatric conditions, prior (or concurrent) cancer therapy of any form within 30 days before POH exposure, current tamoxifen as a chemopreventive agent, concurrent hormone replacement therapy, pregnancy, and life expectancy <1 year.

The study initially targeted premenopausal women because of a parallel study in post-menopausal women. Seven post-menopausal patients of 112-screened women (6%) were enrolled to a parallel Institutional Review Board-approved study in which the aromatase inhibitor exemestane was administered. Exemestane (25 mg orally/day) was administered to four of the seven women for 2 to 14 days. Despite substantial efforts, accrual to this study was inadequate, and it was discontinued prematurely. Because of the small sample size, statistical analysis could not be done, and the data from these patients will not be further discussed in this report.

**Study Design**

POH, formulated into gelatin capsules containing 250 mg of POH and 250 mg of soybean oil, was provided by the Cancer Prevention Branch of the National Cancer Institute (Bethesda, MD). The doses (1.2 to 4.8 g/m²/day) were within the safe range established in animal and human phase I studies (22). To investigate the safety in the perioperative setting for the administration of a single dose of POH, five subjects received a single dose of POH (1.5 g/m²) 2 days before surgery. To further evaluate the level of POH toxicity in the immediate preoperative period, 12 subjects were subsequently scheduled to participate in a dose escalation scheme to obtain a maximum-tolerated dose for prevention. For some cohorts, additional patients were added to ensure safety (Table 1) so that ultimately 16 subjects were exposed to escalating doses of POH. An exploratory end point was to assess possible changes in candidate SEB after short-
term administration of POH. A subsequent plan to expose patients for periods >2 days at the maximum-tolerated dose for prevention was deferred until the activation of an adequately powered follow-up phase II study. Study subjects who met the eligibility criteria but who declined POH treatment were used as controls (patient’s choice) as long as they had provided a written consent that their biopsies and re-excision tissues could be used for research.

Study Assessment

The tolerability of POH was assessed by self-reporting and by questioning from the study coordinator. Toxicity evaluations based on the National Cancer Institute Common Toxicity Criteria (CTCAE version 2.0) were completed at each dose level before a decision to accrue more patients at the next dose level. Patients could be removed from the study for any adverse event, moderate toxicity (grade 2), not responding to dose reduction, noncompliance, patient’s decision to withdraw from the study, and loss to follow-up.

Molecular Biomarkers

Tissue Handling and Preparation. Tissues were obtained from each study subject’s diagnostic biopsy and subsequent excision or re-excision surgery (the subsequent surgery will be designated “re-excision” in the remainder of this report). A consistent method of sample handling was not attempted, because baseline samples were obtained from different centers. Generally, formalin-fixed, paraffin-embedded blocks were available. However, a few patients had their diagnostic biopsies at a different institution, and only immunohistochemistry-ready slides were available. All patients had their re-excision surgical procedure at the Georgetown University Medical Center. A precision microtome (Leitz 1512, Ernst Leitz, Vienna, Austria) was used to prepare 5-μm sections on coated slides (Probe On Plus, Fisher Scientific, Pittsburgh, PA) for each specimen, and slides were refrigerated until use.

Immunohistochemistry and Apoptosis Assays. We selected SEBs that reflected generic proliferation or cell death (Ki67, terminal deoxynucleotidyl transferase-mediated nick end labeling, morphology), tumor differentiation (ER, progesterone receptor, morphology), or important components of pathways modulated by POH in preclinical studies (M6P/IGF2R, TGFβ1; refs. 13, 15, 23, 24).

Slides were deparaffinized in xylene, rehydrated with graded alcohols, treated with 3.0% H2O2, and attached to disposable chambers. All specimens were either blocked with normal goat serum for 20 minutes or 1× Power Block for 8 minutes (BioGenex, San Ramon, CA). Hybridization to primary antibodies was for 1 hour at room temperature. Wash steps during slide preparation used 1× PBS (pH 7.4), with Cadenza buffer containing a surfactant. Antibody localization was visualized with standard biotin-avidin complex methodology (25). The monoclonal antibodies against the following proteins were used: ER (clone ER1D5, dilution 1:50, Immunotec/Coulter, Marseilles, France), progesterone receptor (clone PR1A6, dilution 1:25, Immunotec/Coulter) and Ki67 (clone 7B11, dilution 1:50, Cell Marque, Rockland, CA). The monoclonal antibody against the negative control was anti-M6P/IGF2R antibody (clone 7B11, dilution 1:50, Zymed Lab. Inc., San Francisco, CA).

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Scoring of SEB. A breast pathologist (B. S.) and a technician reviewed H&E staining done on every specimen without knowledge of treatment assignment or tissue pairings. SEB were assessed in areas of normal, proliferative, and malignant breast epithelium (invasive breast cancer and DCIS) common to both the paired diagnostic and re-excision tissue. For each biomarker at least 200 cells were assessed unless <200 were present, in which case every suitable cell was assessed. ER and progesterone receptor were scored with the system described by Allred, which records both frequency and intensity of staining (26). The Allred score is considered negative if the score was 0 or 2 and positive when the score was 3 to 8. The proliferation rate was calculated by counting and dividing the number of Ki67-positive cells by the total number of cells in a representative area and expressed as a percentage. Apoptosis rate was calculated by...
counting and dividing the number of apoptotic cells by the total number of cells seen per light microscopy field at 400× magnification expressed as a percentage. A four-tiered intensity scoring system (0–4) was used to score staining for TGFβ1 and M6P/IGF2R.

Circulating Markers. Serum samples for measurement of IGF-1, IGF-2, and TGFβ1 were drawn before and at the time of definitive surgery. Whenever possible, a sample was drawn during follow-up. All samples were stored at −80°C before analysis. Samples were thawed and assayed with quantitative sandwich immunoassay kits according to the manufacturer’s directions (for IGF-1 and IGF-2, Diagnostic Systems Laboratories, Webster, TX; for TGFβ1, R & D Systems, Minneapolis, MN).

Statistical Considerations

The main objective of this study was to determine feasibility and to establish the logistics related to accrual, collection of tissue, and performance of SEB assays for this novel study design. In this regard, descriptive data are presented with no statistical analysis. A second objective was to evaluate tolerability of POH at various doses, as described in Table 1. An exploratory objective was to determine whether changes in the various SEB could be detected in tissues collected after a short course of POH, relative to matched pretreatment tissues and to serial tissue changes in tissue from control subjects who did not take POH. Because of the pilot nature of the study, this objective was not designed to achieve high statistical power.

Three experimental groups were considered: control (no POH), low-dose (single dose of 1.5 g/m² POH orally two days before surgery, or 1.2 g/m² once a day or twice per day for two days), or high-dose (two days of POH at 1.2 g/m² three times per day or four times per day). For each SEB, we compared the mean change from baseline (i.e., post-treatment versus pretreatment) in the two treatment groups and control group using ANOVA or unpaired t tests (for pair-wise comparisons). The sample size calculation was based on comparing the treated groups versus control with respect to the mean change in biomarker expression. Thus, the power calculation was based on an unpaired t test, with variances adjusted to account for taking the means of pre-post differences (27). Although it was likely that pretreatment and post-treatment measures would be strongly correlated, we based our sample size calculations on a moderate degree of correlation, i.e., \( P = 0.30 \) (resulting in a larger variance). Because of the absence of preliminary data, we expressed the size of the change in terms of units of SD. With 20 patients each in the control and treated groups, power was calculated to be 0.80 to detect a change in mean SEB level of 1 SD, and 0.94 to detect a change of 1.3 SD (considered to be moderate-sized and large-sized effects, respectively). In this small pilot study, we were only interested in discerning effects in the direction of reduced risk; therefore, a one-sided power calculation was sufficient. Ultimately, 21 patients received POH. Potential chemopreventive activity, as measured by changes in SEB, was analyzed with the Mantel-Haenzel \( \chi^2 \) test.

RESULTS

Accrual. Between May 1999 and December 2000, 267 women with a breast biopsy containing neoplasia were screened, but only 37 (14%) participated in the clinical trial (Fig. 1). Nonparticipation was because of protocol ineligibility (30%), failure of patient or her physician to provide information to the study coordinator (22%), surgery scheduling conflict (20%), patient refusal (18%), and miscellaneous logistical issues (10%).

Of the 37 women who participated in the study, 21 were treated with POH. Five women received a single dose (1.5 g/m²) of oral POH 2 days before surgery, and 16 received variable dosages (1.2 g/m² once a day, 1.2 g/m² twice per day, 1.2 g/m² three times per day, or 1.2 g/m² four times per day for 2 days) of POH administered such that the final dose was ingested the evening before surgery (Table 1). Sixteen women agreed to participate in the study but declined POH and served as controls. Formalin-fixed, paraffin-embedded diagnostic and re-excision tissue were available on all 37 patients enrolled.

Toxicity of POH. POH was well tolerated in all patients. Mild (all National Cancer Institute Common Toxicity Criteria grade 1) side effects occurred in six patients, with gastrointestinal reflux being the most frequent (Table 2). Side effects were no more frequent at the higher dose range of POH. With the exception of a transiently elevated white cell count and bilirubin in one patient who was on 1.2 g/m² four times per day for 2 days, hematologic and biochemical measurements remained within normal range in all patients at all dose levels. No dose reduction or drug discontinuation was required for any of the study participants.

Available Tissue for SEB Analysis. To maximize accrual, patients were eligible if they had any type of diagnostic biopsy, except for a fine needle aspiration, as long as the margins were positive and a subsequent definitive re-excision was required. The agreement between the pathology in the diagnostic and re-excision biopsies is provided in Table 3. Infiltrating cancer was the most common proliferative lesion, followed by DCIS. Of the five patients who were administered a single dose POH, only two had neoplastic tissue in both the biopsy and re-excision specimens. In the dose escalation group, eight patients (47%) had the same pathologic lesion in the baseline biopsy and re-excision surgical specimens. Of the patients that were recruited into the control group, seven (47%) had the same lesion in paired specimens. Six of the seven core needle diagnostic biopsies contained infiltrating ductal carcinoma, one contained DCIS, and one contained infiltrating ductal carcinoma and infiltrating lobular carcinoma. Of these, six subsequent re-excision specimens contained the same lesion, and one woman received preoperative chemotherapy and did not undergo a subsequent surgery. Thus, the agreement between baseline and subsequent pathology in women who had a core diagnostic biopsy was 100%. Of the 29 women who had excisional biopsies there was a 45% agreement with the definitive surgical specimen.

Modulation of SEB. ANOVA or an unpaired t test (for comparisons between two groups at a time) used to compare the mean treatment effect (i.e., the post-treatment value minus the pretreatment value) among the following three groups: (a) controls (prospective controls only), (b) low-dose, including single-dose POH and the first 2 dose escalation groups, and (c) high-dose, including the 3rd and 4th dose escalation groups. We did not observe statistically significant differences in the mean change among all three groups or between the high-dose and...
control groups for all SEB (Table 4). Differences were also not significant for comparison of both treatment groups combined versus prospective controls (data not shown).

For each variable, Table 4 shows the mean change and sample size in each experimental group, the $P$ value for the ANOVA comparison of all three groups, and the $P$ value for the comparison of high-dose versus control. Overall, the analyses are limited by the small sample sizes. Most of the analyses included only three to four patients in each of the low- and high-treatment groups. Variability tended to be large; in most cases the SD was at least twice as large and often larger than the mean difference between pre- and post-treatment values. Because assays could not be done on all samples for each candidate SEB, and variance was large relative to changes in SEBs, the nominal power calculated before the study assuming 20 subjects/group was not achieved for this exploratory objective.

**DISCUSSION**

The main aim of this pilot study was to determine the feasibility of conducting a phase II trial in which a novel agent was administered before surgical re-excision of premalignant or malignant breast disease. Secondary aims were to determine tolerability of a short-term preoperative administration of POH. An exploratory objective was to generate evidence that the natural compound POH might have chemopreventive activity in breast tissue by evaluating candidate SEB modulation. Women with a new diagnosis of breast neoplasia were asked to partic-

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**Table 2** Documented adverse events in POH pilot trial

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>No. of patients *</th>
<th>NCI grade</th>
<th>Relation to drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal reflux</td>
<td>5</td>
<td>1</td>
<td>Probable</td>
</tr>
<tr>
<td>Elevated bilirubin</td>
<td>1</td>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>Elevated white cell count</td>
<td>1</td>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>Hot flash</td>
<td>1</td>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>Erythema</td>
<td>1</td>
<td>1</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
<td>1</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

Abbreviation: NCI, National Cancer Institute.

* $N = 6$. Several side effects were reported in one patient.
Table 4  SEB modulation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control *</th>
<th>Low dose</th>
<th>High dose</th>
<th>High versus low versus control P</th>
<th>High versus control P</th>
</tr>
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<tbody>
<tr>
<td>Elston grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>−0.25 (4)</td>
<td>1.00 (4)</td>
<td>−0.50 (4)</td>
<td>0.32</td>
<td>0.73</td>
</tr>
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<td>Cancer</td>
<td>−1.20 (5)</td>
<td>−2.75 (4)</td>
<td>1.33 (3)</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td>CIS</td>
<td>−3.00 (3)</td>
<td>0.00 (4)</td>
<td>1.00 (1)</td>
<td>0.33</td>
<td>ND</td>
</tr>
<tr>
<td>PgR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.39 (13)</td>
<td>−0.57 (7)</td>
<td>1.83 (6)</td>
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<td>0.25</td>
</tr>
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<td>Cancer</td>
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<td>−5.00 (1)</td>
<td>0.15</td>
<td>ND</td>
</tr>
<tr>
<td>CIS</td>
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<td>−1.00 (4)</td>
<td>8.00 (1)</td>
<td>0.12</td>
<td>ND</td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>−4.00 (5)</td>
<td>0.67 (3)</td>
<td>−2.00 (2)</td>
<td>0.21</td>
<td>0.52</td>
</tr>
<tr>
<td>Cancer</td>
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</tr>
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<td>0.17</td>
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</tr>
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<td>0.00 (3)</td>
<td>0.00 (1)</td>
<td>0.61</td>
<td>ND</td>
</tr>
<tr>
<td>Cancer</td>
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<td>0.00 (3)</td>
<td>0.00 (1)</td>
<td>0.61</td>
<td>ND</td>
</tr>
<tr>
<td>CIS</td>
<td>0.00 (5)</td>
<td>0.00 (1)</td>
<td>0.00 (2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TGFβ1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.57 (7)</td>
<td>0.33 (3)</td>
<td>−0.50 (4)</td>
<td>0.46</td>
<td>0.27</td>
</tr>
<tr>
<td>Cancer</td>
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<td>0.50 (4)</td>
<td>0.33 (3)</td>
<td>0.75</td>
<td>0.69</td>
</tr>
<tr>
<td>CIS</td>
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<td>−0.50 (2)</td>
<td>1.00 (1)</td>
<td>0.13</td>
<td>ND</td>
</tr>
<tr>
<td>TGFβ2</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Normal</td>
<td>−0.18 (11)</td>
<td>0.00 (6)</td>
<td>−0.20 (5)</td>
<td>0.87</td>
<td>0.96</td>
</tr>
<tr>
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<td>−0.25 (4)</td>
<td>0.00 (2)</td>
<td>0.45</td>
<td>0.27</td>
</tr>
<tr>
<td>CIS</td>
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<td>0.00 (2)</td>
<td>−1.00 (1)</td>
<td>0.78</td>
<td>ND</td>
</tr>
<tr>
<td>IGF2R</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Normal</td>
<td>−0.40 (10)</td>
<td>−0.75 (8)</td>
<td>0.00 (5)</td>
<td>0.57</td>
<td>0.55</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.33 (6)</td>
<td>−1.50 (4)</td>
<td>2.00 (1)</td>
<td>0.02</td>
<td>ND</td>
</tr>
<tr>
<td>CIS</td>
<td>−2.00 (1)</td>
<td>0.00 (3)</td>
<td>−1.00 (1)</td>
<td>0.65</td>
<td>ND</td>
</tr>
<tr>
<td>Circulating markers</td>
<td></td>
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<tr>
<td>PIGF1</td>
<td>−1.00 (1)</td>
<td>0.33 (12)</td>
<td>8.13 (8)</td>
<td>0.62</td>
<td>ND</td>
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<tr>
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<tr>
<td>PIGF1</td>
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<tr>
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Abbreviations: CIS, carcinoma in situ; PIGF, plasma insulin growth factor; PTGF, plasma transforming growth factor.

* Number of patients in parenthesis.
imate in a study of POH administration between diagnostic and re-excision surgeries. Of 267 potentially eligible women screened over 18 months, 14% participated in the study. Of the 230 women who were screened but did not enroll in the study, 30% were ineligible, and the remaining subjects were not enrolled because of either failure of either the patient or the surgeon to return calls to the study coordinator after an initial contact to determine eligibility, the timing of scheduled surgery precluded entry into the trial, or other logistical difficulties. For example, the Georgetown University Medical Center is a tertiary referral center with patients referred from a wide geographical area. For some patients it was simply too difficult to arrange transportation for additional study visits in the short time available between procedures. Finally, 18% of patients that were screened declined entry. In these cases, it was the opinion of the study coordinator that patients were reluctant to enter the study if routine and study visits could not be combined or if blood collection would prolong the visit. Eligible patients were anxious about their recent diagnosis and wished to proceed to re-excision as quickly as possible. Moreover, for ethical reasons, it was explained to patients that because the period of chemopreventive administration was very short, it was unlikely that it was explained to patients that because the period of chemopreventive administration was very short, it was unlikely that participation in the pilot study would have any benefit for the individual patient.

Other investigators have also encountered logistical barriers when accruing to perioperative SEB studies. Singletary et al. (28, 29) reported that only 1% of 4,514 screened patients entered a perioperative biomarker trial of a 2- to 4-week treatment with the combination tamoxifen and the retinoid N-(4-hydroxyphenyl)retinamide at M. D. Anderson Comprehensive Cancer Center. The investigators hypothesized that the treatment arm would result in a 20% reduction in Ki67, and placebo would be associated with a 5% reduction in marker; however, similar reductions were observed in the placebo (n = 16) and treatment arms (n = 20). As in our study, the number of study participants did not allow the M. D. Anderson investigators to see meaningful changes in surrogate markers. However, other centers have been more successful. Dowsett et al. (30) successfully compared paired baseline and definitive biopsies of patients who were randomly assigned raloxifene versus placebo for 14 days preoperatively. Raloxifene therapy was associated with a statistically significant reduction in ER and Ki67 in ER-positive but not in ER-negative tumors.

Taken together, the results of our study with those of Dowsett and Singletary show that perioperative studies are feasible but require a dedicated study coordinator, a high number of eligible patients, a pathology report screening system to identify potentially eligible patients, and efficient interactions with surgeons, radiologists, and the study team members to identify patients as early as possible after diagnosis. Even with all of these features are in place in a well-organized comprehensive cancer center, the logistical complications still precluded successful completion of our study as planned in a timely fashion. It is also possible that women are less likely to participate in a study evaluating a novel agent with unknown activity and may be more likely to enroll in a study using an agent with known activity, such as selective estrogen receptor modulators in the study by Dowsett et al.

A second aim of this study was to evaluate the toxicity of short-term administration of POH. At the duration and doses used in this study, POH was well tolerated with only mild side effects reported in a minority of patients. Although duration of therapy in this trial design was short, longer periods of treatment in the context of patients with refractory solid malignancies are also well tolerated (22). Future perioperative study of 1 or 2 weeks POH exposure may be feasible from an agent tolerability standpoint.

An exploratory aim of this study was to determine whether adequate tissue could be collected for satisfactory candidate SEB analysis before and after treatment with a chemopreventive agent. Unfortunately, the agreement between initial biopsy and subsequent surgery was only 56%. Therefore, meaningful assessment of SEB could not be completed. Absence of residual cancer in re-excision of specimens with positive margins is expected in up to 75% of cases (31) so it is not surprising that the most common discrepancy was a lack of neoplastic tissue in the re-excision specimen. If patients with positive margins of excision remain eligible in future perioperative studies, researchers should consider enrolling a larger sample size or limiting eligibility to patients with residual disease on imaging studies to ensure an adequate number of paired samples to make definitive conclusions about SEB modulation. Inclusion of women with ductal carcinoma in situ or invasive cancer and positive margins but not women with premalignant lesions is also more likely to result in additional tissue in re-excision specimens.

Another approach would be to follow and focus on patients who have undergone a diagnostic core needle biopsy and are scheduled for their first surgical resection. In this instance it is much more likely that the same index lesion will be captured before and after exposure to the chemopreventive agent. However, one must be aware that agreement of SEB measurements between core biopsy and re-excision tissue specimens is not uniform for all markers (32). Enrollment of patients with a baseline core biopsy may also allow for the accrual of frozen tissue for gene expression profiling. We have reported previously our successful experience of conducting gene expression arrays on core needle biopsy material obtained during diagnostic procedures (33). As long as care is taken to ensure that the experimental biopsies are screened for diagnostic information (by frozen section H&E) in a timely manner, these extra SEB biopsies should not interfere with patient management.

Candidate SEB analyses were done as outlined in the protocol. Although assays were performed for morphology, proliferation, ER, progesterone receptor, and apoptosis on normal, proliferative, and malignant epithelium, the sample size for each marker was too small to observe consistent trends in tissue SEB either in the single dose or at the dose escalation levels. This negative result should not be regarded as suggesting lack of activity of POH on hyperplastic or malignant breast tissue because this aim was exploratory and had low power to detect anything but large changes in SEBs. The low power of this pilot objective of the study enhanced the likelihood that a real change could not be detected with sufficient precision to achieve statistical significance. An additional factor contributing to the lack of significant changes in the SEB outcomes may have been the low-dose and short duration of POH exposure necessitated by...
the dose escalation schema. Only an adequately powered SEB analysis of early breast lesions after similarly "long" exposure to the 1.2 g/m² four times per day dose would be sufficient to provide robust positive or negative information on the likely chemopreventive activity of POH in breast cancer. However in a phase I study, 14 days of POH were not associated with a consistent modulation of p21ras, rap1, or rhoA in peripheral blood mononuclear cells (22). Finally, it will be important to develop a more concentrated formulation of POH for future studies because women at the highest dose level in this study had to ingest up to 38 capsules/day.

Our study showed that short-term perioperative SEB studies to confirm the potential chemopreventive activity of novel compounds are possible as long as the agent has a rapid onset of action, a low toxicity profile, and a dedicated team is available to efficiently screen large numbers of potential subjects for eligibility. Patients are asked to participate in a perioperative clinical trial that provides them with no benefit at a time when anxiety levels are high and preparations for surgery were underway. We recommend focusing on women with a diagnosis of premalignant or malignant breast lesion by core needle biopsy to maximize the chance of obtaining paired biopsies for SEB analysis and to acquire tissue samples for nucleic acid based genomic analysis. Alternatively, serial collection of normal breast ductal epithelium by fine needle aspiration, core needle biopsy, or ductal lavage, or marker assessment in secreted fluid or serum or urine, may be more productive and successful strategies to assess SEBs in phase II studies of putative chemopreventive agents (34–36).

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A Pilot Surrogate End Point Biomarker Trial of Perillyl Alcohol in Breast Neoplasia

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