Research Article

Cancer Prevention Research

Phase IB Randomized, Double-Blinded, Placebo-Controlled, Dose Escalation Study of Polyphenon E in Women with Hormone Receptor–Negative Breast Cancer

Katherine D. Crew[1], Powel Brown[3], Heather Greenlee[1], Therese B. Bevers[3], Banu Arun[3], Clifford Hudis[2], Heather L. McArthur[2], Jenny Chang[4], Mothaffar Rimawi[5], Lana Vornik[3], Terri L. Cornelison[6], Antai Wang[1], Hanina Hibshoosh[1], Aqeel Ahmed[1], Mary Beth Terry[1], Regina M. Santella[1], Scott M. Lippman[3], and Dawn L. Hershman[1]

Abstract

Epidemiologic data support an inverse association between green tea intake and breast cancer risk, and numerous experimental studies have shown the antitumor effects of its main component, epigallocatechin gallate (EGCG). We conducted a phase IB dose escalation trial in women with a history of stage I to III hormone receptor–negative breast cancer of an oral green tea extract, polyphenon E (Poly E) 400, 600, 800 twice daily or matching placebo for 6 months. The primary endpoint was to determine the maximum tolerated dose (MTD), defined as the dose that causes 25% dose-limiting toxicity (DLT, grade ≥II). Assignment to dose level was based upon an adaptive design, the continual reassessment method. A mammogram and random core biopsy of the contralateral breast were obtained at baseline and 6 months and serial blood/urine collections every 2 months for biomarker analyses. Forty women were randomized: 10 to placebo, 30 to Poly E (16 at 400 mg, 11 at 600 mg, 3 at 800 mg). There was one DLT at 400 mg (grade III rectal bleeding), three DLTs at 600 mg (grade II weight gain, grade III indigestion and insomnia), and one DLT at 800 mg (grade III liver function abnormality). The DLT rate at 600 mg was 27% (3 of 11). Pharmacologic levels of total urinary tea polyphenols were achieved with all three dose levels of Poly E. Using a novel phase I trial design, we determined the MTD for Poly E to be 600 mg twice daily. This study highlights the importance of assessing toxicity for any chemopreventive agent being developed for chronic use in healthy individuals. *Cancer Prev Res; 5(9); 1144–54. ©2012 AACR.*

Introduction

Chemoprevention with selective estrogen receptor modulators (SERM), tamoxifen (1) and raloxifene (2), or the aromatase inhibitor, exemestane (3), reduces breast cancer incidence among high-risk women. However, uptake has been poor in the prevention setting. In addition, these agents have no effect on the incidence of hormone receptor (HR)-negative cancers, which account for about a third of all breast carcinomas and are associated with a poorer prognosis. The high costs of large-scale chemoprevention studies have prompted the search for intermediate markers of cancer development. A greater emphasis has been placed on developing novel clinical trial designs which use surrogate endpoint biomarkers in lieu of cancer occurrence to improve the efficiency and reduce the cost of chemoprevention trials. Therefore, priorities in breast cancer chemoprevention include developing safe and tolerable agents that are effective against HR-negative breast cancer and validating intermediate biomarkers which correlate with breast cancer risk.

Breast cancer is the most common cancer among women worldwide, with a 6-fold variation in incidence between high-risk regions (e.g., North America and Europe) and low-risk regions (e.g., Asia; ref. 4). This geographic variation is often attributed to the Asian diet, which is rich in soy-based products and antioxidant-containing foods and beverages, such as green tea. A recent meta-analysis, encompassing 5,617 breast cancer cases, reported an inverse association between green tea consumption and breast cancer incidence [relative risk (RR), 0.81; 95% confidence interval (CI), 0.75–0.88] among case–control studies (5). In 2 Japanese cohort studies (6, 7), green tea intake among patients with

Authors' Affiliations: [1]Herbert Irving Comprehensive Cancer Center, Columbia University; [2]Memorial Sloan-Kettering Cancer Center, New York; [3]University of Texas, MD Anderson Cancer Center; [4]The Methodist Hospital Cancer Center; [5]Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, Texas; and [6]Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

ClinicalTrials.gov number: NCT00516243.

Corresponding Author: Katherine D. Crew, Herbert Irving Comprehensive Cancer Center, Columbia University, 161 Fort Washington Ave, 10-1072, New York, NY 10032. Phone: 212-305-1732; Fax: 212-305-0178; E-mail: kd59@columbia.edu

doi: 10.1158/1940-6207.CAPR-12-0117

breast cancer was associated with a decrease in risk of recurrence (pooled RR, 0.73; 95% CI, 0.56–0.96). A population-based, case–control study of Asian American women found that green tea intake was associated with decreased breast cancer risk with more than 85.7 mL/d (OR, 0.53; 95% CI, 0.35–0.78; ref. 8). Interestingly, the protective effect of green tea was observed only among individuals who possessed at least one low-activity polymorphism in catechol-*O*-methyltransferase (*COMT*), which is involved in the methylation of green tea constituents (9).

The most abundant and possibly most potent polyphenol in green tea is epigallocatechin-3-gallate (EGCG; ref. 10). EGCG has anti-oxidative, anti-inflammatory, antiproliferative, and antiangiogenic properties that are relevant for cancer prevention (11). *In vitro* studies have shown cytotoxic effects of EGCG on breast cancer cells regardless of estrogen receptor (ER) status (12). A major challenge is translating these preclinical findings into human intervention trials.

Polyphenon E (Poly E) is a well-defined pharmaceutical-grade decaffeinated green tea catechin mixture, including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and most abundantly, approximately 65% EGCG (13). Each capsule contains 200 mg of EGCG, which is equivalent to about 2 to 3 cups of brewed green tea. In phase I pharmacokinetic trials of Poly E 200 to 800 mg daily (equivalent to 2–12 cups of green tea without caffeine) given as a single-dose or 4-week administration in healthy individuals, high plasma EGCG levels were achieved (14, 15). All adverse events were rated as mild, including nausea, abdominal pain, heartburn, excess gas, headache, dizziness, and muscle pain (15).

We conducted a phase IB randomized, double-blinded, placebo-controlled, dose escalation trial of a 6-month intervention of Poly E in women with a history of HR-negative breast cancer. Women with HR-negative breast cancer provide a relevant population for conducting secondary prevention research because they are not candidates for adjuvant hormonal therapy and may be particularly motivated to participate in chemoprevention trials. The primary objective of this dose-finding study was to determine the maximum tolerated dose (MTD) of Poly E given over a 6-month period. Secondary objectives were to determine the dose-related biologic effects of Poly E on intermediate biomarkers correlated with breast cancer risk, including breast tissue–based biomarkers, mammographic density, and serum hormone levels, as well as *COMT* genotype and urinary tea polyphenols.

Subjects and Methods

Subjects

Women were enrolled at 4 sites: Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; University of Texas MD Anderson Cancer Center, Houston, TX; Memorial Sloan-Kettering Cancer Center, New York, NY; Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX. The study was approved by the institutional review boards at each participating site. All participants provided written informed consent in English or Spanish. The study was registered before initiating enrollment (http://clinicaltrials.gov, NCT00516243).

Eligible participants were women ages 21 to 65 years with a history of histologically confirmed resected stage I–III ER-negative and progesterone receptor (PR)-negative (defined as <10% ER and PR expression) breast carcinoma without evidence of disease at trial entry and a minimum of 6 months since completion of breast surgery, adjuvant chemotherapy (including trastuzumab), and radiation therapy. Other eligibility criteria included an Eastern Cooperative Group performance status of 0 to 1, at least one intact breast (no radiation therapy, mastectomy, or breast implant), and normal organ and marrow function, including total bilirubin and transaminases within normal institutional limits.

Exclusion criteria included bilateral breast cancer or metastatic disease, history of gastrointestinal bleeding, uncontrolled or significant comorbid illness, and current use of hormone replacement therapy, tamoxifen, or raloxifene. Both pre- and postmenopausal women were included in this study. Postmenopausal status was defined as the absence of menses for more than 12 months, history of bilateral oophorectomy, or serum follicle-stimulating hormone (FSH) more than 20 mIU/mL.

Participants had to abstain from all tea consumption (including herbs, vitamin, and mineral supplements that contain tea compounds) and limit total daily caffeine consumption to less than 375 mg for 30 days before the baseline evaluation and during the 6-month study intervention.

Trial design and intervention

The trial was a randomized, double-blinded, placebo-controlled, dose escalation study in which participants received either Poly E delivering 400, 600, or 800 mg of EGCG (2–4 capsules) twice daily (total of 800, 1,200, or 1,600 mg EGCG daily) with food or matching placebo for 6 months (10 subjects in the placebo arm, 30 subjects in the study arm with participant assignment to dose level as per the adaptive study design). The Poly E oral capsules and matching placebo were supplied by the National Cancer Institute (NCI), Division of Cancer Prevention (DCP) under an IND. Participants and investigators were blinded to Poly E or placebo but not to dose level.

The primary objective of this phase I dose escalation study was to determine the MTD of Poly E in this study population. All participants were evaluable for toxicity from the time of their first dose of study drug. Safety was assessed by monitoring routine clinical and laboratory parameters at baseline, every 2 weeks during the first month, then monthly for the duration of the study. The starting dose of Poly E 400 mg twice daily was chosen on the basis of previous clinical safety data (14, 15). The MTD was defined as a dose that causes a dose-limiting toxicity (DLT) in approximately 25% of participants during the 6-month intervention. A DLT was initially defined according to the

NCI Common Terminology Criteria for Adverse Events (Version 3.0) as any grade II or higher toxicity. After the first 2 grade II toxicities occurred during the trial, the protocol was amended to define the DLT as any grade II or higher toxicity which was at least possibly related to study drug, persisted for at least 1 week or required stopping the study drug. A lower threshold for a DLT was used compared with cancer treatment trials, as Poly E is an agent being developed for chemoprevention where prolonged use can be anticipated.

The time-to-event continual reassessment method (TITE-CRM) is a novel statistical methodology that considers long-term toxicity in dose escalation, allows staggered participant entry, and on average allocates more participants at the correct MTD (16). According to the TITE-CRM, the decision for escalation was made after every 5 subjects treated with Poly E were evaluated for toxicity at a single dose. Dose escalation took place with 5 nontoxic observations. Once a DLT was observed, the dose toxicity curves were re-assessed, and the next group of 5 was given a dose according to the TITE-CRM. Once a DLT occurred at any time in the 6-month window in any subject, new subjects were considered at the most updated MTD estimate. A participant who developed a DLT was taken off of study drug but was encouraged to complete the 6-month evaluations. There were no intraparticipant dose modifications.

Correlative studies

Secondary objectives of this study were to investigate the dose-related biologic effects of Poly E. Participants underwent a digital mammogram and random core biopsy of the contralateral breast at baseline and after the 6-month intervention for mammographic density assessment and immunohistochemical (IHC) analyses of Ki-67 (proliferation marker) and ER-α. Because of changes in breast density and breast tissue proliferation with the menstrual cycle, mammograms and breast biopsies were obtained during days 7 to 14 of the menstrual cycle in premenopausal women. Mammographic percent density (proportion of the breast with dense tissue) from the craniocaudal view of the contralateral breast was assessed using semi-automated methods by the Cumulus software (17). All mammographic density readings were conducted by an investigator blinded to treatment assignment and the timing of the mammograms (baseline or 6 months). A random core biopsy from the top outer quadrant of the contralateral breast approximately 1 cm from the areolar edge was obtained at baseline and 6 months. Tissue was placed in a vial of 10% formalin for a maximum of 24 hours before embedding and processing. IHC analysis of Ki-67 (Clones MIB-1 and M7240 mouse monoclonal, 1:50; Dako) and ER-α (Clones 1D5 and M7047 mouse monoclonal, 1:60; Dako) was conducted on paraffin-embedded sections. Quantitative changes of the expression of markers based upon percentage of positive cells were scored in a blinded fashion.

Blood samples were collected at baseline, 2, 4, and 6 months for measurement of serum hormone levels: estradiol, testosterone, insulin-like growth factor-1 (IGF-1), IGF-binding protein-3 (IGFBP-3), and sex hormone–binding globulin (SHBG). Radioimmunoassays were used to measure serum estradiol and testosterone levels (Diagnostic Systems Laboratories). The lowest limit of detection for estradiol and testosterone were 5.0 pg/mL and 20 ng/dL, respectively. Assays for IGF-1, IGFBP-3, and SHBG were immunometric using enzyme/substrate for detection (Diagnostic Systems Laboratories).

Spot urine samples were collected every 2 months during the 6-month intervention. Five metabolites of tea catechins [EGC, EC, methylepigallocatechin (4′-MeEGC), 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4), and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6)] were assayed by high-performance liquid chromatography, which allows the determination of free and conjugated forms of tea catechins in urine, as previously described (18). The lower limit of detection for each catechin was 0.02 μmol/L. For each subject, we summed the EGC, EC, 4′-MeEGC, M4, and M6 levels to create a total tea polyphenols index. The concentration of total tea polyphenols was expressed in units of urinary creatinine by weight (μmol/g Cr) to account for varying volumes between the spot urine samples.

Genomic DNA was extracted from blood cells (10 mL/sample) taken at baseline using standard RNase/proteinase K technique and genotyping for *COMT* (G/A transition at codon 158, rs4680) was conducted using the TaqMan 5′-Nuclease Assay (Applied Biosystems). The fluorescence profile of each well was measured in an ABI 7500HT Sequence Detection System, and the results were analyzed with Sequence Detection Software (Applied Biosystems).

A questionnaire on quality of life (SF-36) was self-administered at baseline and 6 months. The short form of the Medical Outcomes Study (SF-36) is composed of 36 items across 8 scales: physical functioning, role function—physical, bodily pain, general health, vitality, social functioning, role function—emotional, and mental health (19).

Statistical considerations

Before the actual implementation of the trial, computer simulations were used to evaluate the operating characteristics of the TITE-CRM approach to dose assignment (Table 1). A robust dose toxicity model was calibrated according to Cheung and Chappell (20). Overall, the design would be able to identify the correct MTD with a probability over 0.60 when the neighboring doses had DLT rates of 10% and 35%, respectively. If the neighboring doses had DLT rates significantly different from the target of 25%, the probability of correct selection of the MTD would be higher. The TITE-CRM has been shown to generally yield a high probability of selecting the correct MTD even with a small sample size. Only the 30 participants receiving active study drug participated in the dose escalation study. In principle, as few as 12 participants could be recruited with the TITE-CRM but having at least 10 participants at the MTD would avoid too much variability in the comparisons to placebo.

Table 1. *A priori* operating characteristics under each scene based upon 5,000 computer simulations

Dose	400 mg	600 mg	800 mg	Average no. of DLTs
Scene 1: 400 mg is the MTD				
DLT rate	**25%**	35%	35%	
P (SELECT)	**0.73**	0.23	0.04	8.5
Average no. of allocations[a]	20	8	2	
Scene 2: 600 mg is the MTD				
DLT rate	10%	**25%**	35%	
P (SELECT)	0.13	**0.64**	0.22	6.6
Average no. of allocations	10	14	6	
Scene 3: 800 mg is the MTD				
DLT rate	5%	10%	**25%**	
P (SELECT)	0.00	0.19	**0.80**	4.7
Average no. of allocations	6	10	14	

NOTE: Numbers in bold indicate the prespecified target DLT rate (25%) and probability of selecting that dose as the correct MTD.
Abbreviation: *P* (SELECT), probability of selecting that dose as the MTD.
[a]Average number of participants allocated to each dose level.

Assuming a 10% dropout rate, 36 of the 40 participants would be evaluable for the long-term toxicity and efficacy endpoints. This sample size would ensure that estimates of any binary variable, including incidence of toxicity, would have a 95% CI of width less than 0.36. All participants who received any study drug were included in the report of toxicities. The percentage of participants with each toxicity was compared between the intervention groups using the Fisher exact test of proportions at a 2-sided 0.05 level of significance.

Of the 40 participants, 28 completed the drug intervention (8 placebo, 20 Poly E) and 34 completed their 6-month assessments (8 placebo, 26 Poly E) and were included in the secondary endpoint analyses. There were missing data for some of the endpoints due to inability to collect some of the specimens or inadequate samples for biomarker analysis. The missing rates of the submitted biospecimens were 1.4% for serum/urine biomarkers, 2.5% for *COMT* genotyping, 9% for mammographic density readings, and 12% for IHC tissue biomarkers. Descriptive statistics were conducted on each of the biomarker endpoints within each intervention group. Because of skewness in the distributions of these variables, Wilcoxon signed-rank tests were used to compare between-group and within-group differences for the Poly E and placebo groups for each of the outcome measures using a continuous scale. We also calculated the mean absolute change and percentage change from baseline for each group to account for differences in baseline measures. Between-group comparison for continuous data was conducted using repeated-measure ANOVA with a time interaction term using treatment and dose levels as main effects. These secondary endpoints were not corrected for multiple comparisons due to their exploratory nature. All analyses were 2-sided and conducted using SAS software version 9.1.3 (SAS Institute).

Results

Participant characteristics

Among 508 women screened, 168 met initial eligibility criteria (Fig. 1). From July 2007 to August 2009, 40 participants were randomized 3:1 to Poly E ($n = 30$; 16 at 400 mg, 11 at 600 mg, 3 at 800 mg) or placebo ($n = 10$). Baseline characteristics are presented in Table 2. Median age was 52 years (range, 36–64). The study population was diverse by race/ethnicity (42% minorities), 75% were postmenopausal, and 74% had a body mass index of 25 kg/m^2 or greater. Over three quarters (78%) of participants had stage I or II breast cancer and the median time since diagnosis was 33 months (range, 10–170). Twenty-eight (70%) women completed the 6-month drug intervention, 3 (8%) were nonadherent (did not complete the 6-month drug intervention), 4 (10%) were lost to follow-up, and 5 (12.5%) developed a DLT (Fig. 1). A total of 34 of 40 (85%) participants had 6-month biomarker data available for analysis.

Toxicities

Figure 2 shows the course of participant flow, including dose escalation and de-escalation in the study. The figure illustrates that after 5 nontoxic observations at the first dose level (Poly E 400 mg twice daily or total of 800 mg EGCG daily), dose escalation occurred. Initial escalation to dose level 3 (Poly E 800 mg twice daily or total of 1,600 mg EGCG daily) occurred before the 2 DLTs occurred at dose level 2 (Poly E 600 mg twice daily or total of 1,200 mg EGCG daily). However, additional participants were assigned to the lower dose groups because the study design is able to account for late toxicities. In total, there were 5 DLTs during the trial (in sequential order): grade II weight gain (day 138 on study drug) and grade III indigestion (day 40) at dose level 2; grade III alanine aminotransferase (ALT) elevation

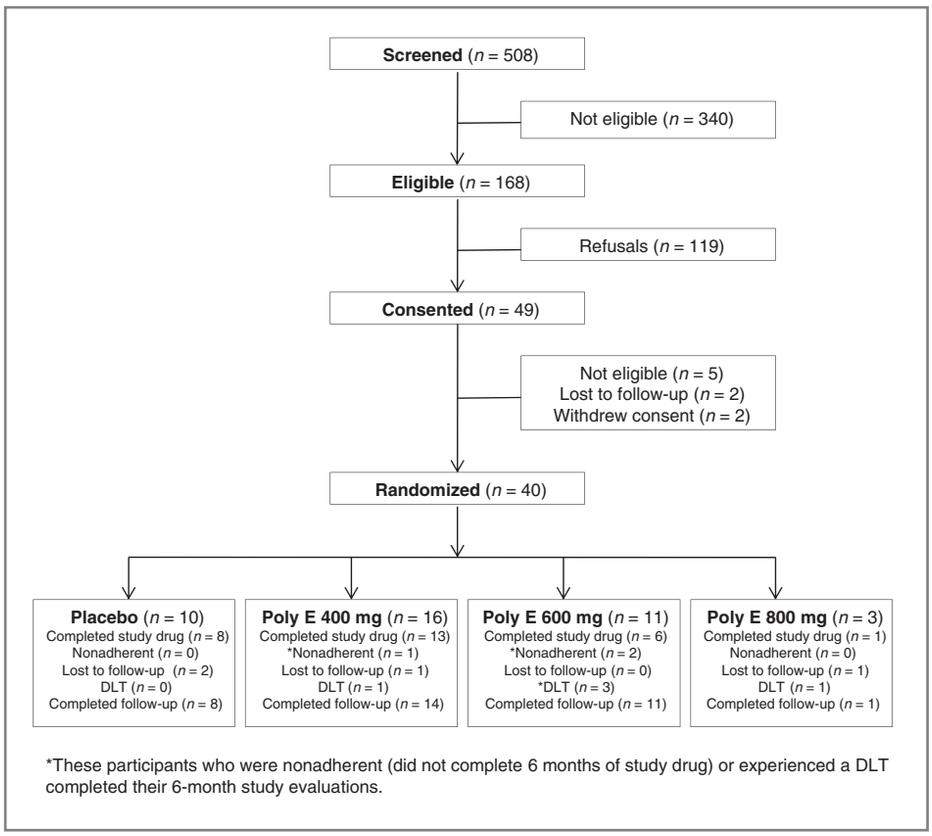

Figure 1. Flow diagram for subjects who were accrued into the study.

(day 91) at dose level 3; grade III insomnia (day 6) at dose level 2; and grade III rectal bleeding (day 18) at dose level 1. The episode of grade III rectal bleeding occurred in a woman with pre-existing diverticulosis and required hospitalization. After this serious adverse event, the protocol was amended to exclude women with a prior history of a gastrointestinal bleed. All DLTs occurred in participants receiving active Poly E. The frequency of DLTs was 6.25% (1 of 16) for dose level 1, 27% (3 of 11) for dose level 2, and 33% (1 of 3) for dose level 3. These DLT rates are similar to what was predicted with the computer simulations (Table 1). On the basis of these findings, dose level 2 (600 mg twice daily) was defined as the MTD for Poly E because it was the closest to the prespecified toxicity level of 25%.

Additional toxicities are summarized in Table 3. The toxicity profile of Poly E was consistent with the published literature, with the most common adverse events (mainly grade I) being gastrointestinal in nature. There were a handful of grade II toxicities in the Poly E and placebo arms that did not meet criteria for a DLT based upon investigator assessment, as they did not require stopping study drug. The toxicities did not differ significantly by Poly E dose level (data not shown) or compared with placebo.

Biomarker analyses

Secondary biomarker endpoints are summarized in Table 4. No significant trends were observed with the serial blood/urine biomarkers over time (data not shown); therefore, only the baseline and 6 month data are presented. Baseline and 6 month biomarker data were available for 26 participants in the Poly E group and 8 in the placebo group. The Poly E–treated group had a greater increase in mean total urinary tea polyphenols compared to placebo (152.1 $\pm$ 186.4 vs. 11.8 $\pm$ 13.7 μmol/g Cr, $P = 0.001$). However, the levels of urinary tea polyphenols did not differ significantly by dose level of Poly E (data not shown). Because of small numbers, the 3 dose levels of Poly E were combined for all biomarker analyses. There was about a 70% reduction in serum estradiol levels ($P = 0.05$) and a significant decrease in SHBG ($P = 0.03$) at 6 months compared with baseline in the Poly E group. However, these changes did not differ significantly compared with the placebo group, with the magnitude of changes in the placebo arm being greater but not statistically significant due to smaller numbers. Serum IGFBP-3 showed a greater mean increase for those on Poly E relative to placebo but again this did not reach statistical significance (462 $\pm$ 921 vs. 250 $\pm$ 782 ng/mL, $P = 0.91$). The IGF-1/IGFBP-3 ratio decreased by 31% in the Poly E group compared with a 2.5% reduction among those who received placebo ($P = 0.25$).

In terms of target tissue effects, no significant changes in the Ki-67 proliferation index or mammographic density were seen after 6 months of Poly E compared with placebo. These results did not differ when stratified by menopausal status (data not shown). Of note, mean baseline Ki-67 levels

Table 2. Participant characteristics

Characteristics	Poly E ($n = 30$)	Placebo ($n = 10$)	Total ($n = 40$)
Age, y			
Median	52	53.5	52
Range	36–64	40–59	36–64
Menopausal status, *n* (%)			
Premenopausal	7 (23)	3 (30)	10 (25)
Postmenopausal	23 (77)	7 (70)	30 (75)
Race, *n* (%)			
White	18 (60)	5 (50)	23 (58)
Hispanic	6 (20)	3 (30)	9 (22)
Black	5 (17)	2 (20)	7 (18)
Asian	1 (3)	0	1 (2)
Body mass index, kg/m^2			
Median	28.2	28.7	28.6
Range	21.1–40.7	22.6–37.3	21.1–40.7
Breast cancer stage at diagnosis, *n* (%)			
I	14 (47)	2 (20)	16 (40)
II	9 (30)	6 (60)	15 (38)
III	7 (23)	2 (20)	9 (22)
Breast cancer treatments, *n* (%)			
Chemotherapy	29 (97)	10 (100)	39 (98)
Trastuzumab	5 (17)	2 (20)	7 (18)
Radiation therapy	23 (77)	8 (80)	31 (78)
Months since breast cancer diagnosis			
Median	31.5	37	33
Range	10–170	15–73	10–170

in the random core breast biopsies of the women in the Poly E and placebo arms were low (1.5% ± 1.4% and 0.8% ± 1.1%, respectively). There was a favorable but nonsignificant decrease in mean ER-α expression with Poly E and placebo (−4.4% ± 14.9%, $P = 0.23$ vs. −10.2% ± 21.4%, $P = 0.25$). Supplementary Figure S1 provides a graphical representation of these results.

The genotypic distribution of *COMT* among study subjects was *GG*, 28%; *GA*, 54%; and *AA*, 18%. In the Poly E–treated group, mean urinary total tea polyphenol levels did not differ significantly among those who carried at least one high-activity *COMT* allele (*GG* and *GA* genotype) compared with individuals possessing the homozygous variant genotype (*AA*). Finally, no significant change in quality of life as assessed by the SF-36 was observed in either treatment group (data not shown).

Discussion

This study represents a novel approach to conducting a phase I trial evaluating long-term safety and determining the optimal dose of a potential chemopreventive agent, polyphenon E. We encountered 5 DLTs during the trial: 2 occurring within the first month on study drug and the others up to nearly 5 months on study drug. Other toxicities were generally mild and did not differ significantly compared with placebo. Although there were transient increases in liver function tests and pancreatic enzymes, there was no evidence of clinically significant liver or pancreatic disease.

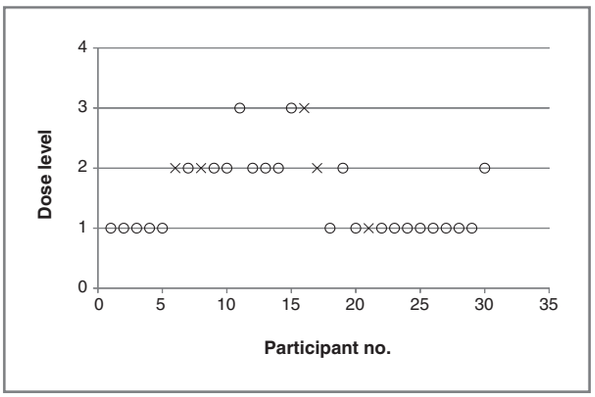

Figure 2. Participants' flow throughout the course of the trial. Participants who received active Poly E ($n = 30$) are presented in chronologic order from left to right with dose levels on the vertical axis (1 = 400 mg twice daily, 2 = 600 mg twice daily, 3 = 800 mg twice daily). Open circles indicate participants who did not develop a DLT and crosses indicate participants who developed a DLT. Note the dose escalations and de-escalations that occurred with ongoing data collection on DLTs. The first participant at dose level 3 was enrolled before the 2 participants at dose level 2 developed a DLT.

The TITE-CRM is a novel statistical methodology that considers long-term (e.g., 6 months) toxicity in dose escalation while allowing staggered participant entry. Therefore, the trial was conducted in a continuous fashion without accrual suspension. The TITE-CRM on average allocates more participants at the correct MTD, thus enhancing comparison between the placebo and the MTD on other secondary endpoints. However, in our study, more women were assigned to the 400-mg dose ($n = 16$) rather than 600-mg dose ($n = 11$). The main advantage of the TITE-CRM is that it allows for the evaluation of long-term toxicities, unlike a conventional dose escalation method with 1 month of observation, which may underestimate late toxicities. Thus, by allowing additional participants to be enrolled at lower dose levels, the TITE-CRM will account for late toxicities that occur after months of treatment, as well as acute toxicity that may appear in the first month. With this trial, we were able to show that an adaptive method of dose escalation that has been used extensively in cancer treatment trials may also be useful in an early-phase chemoprevention trial. An alternative to defining the optimal dose by the MTD (dose with a 25% DLT rate) is to determine the minimal effective dose. One could argue that as there was no difference in urinary tea catechin levels and biomarker effects at the 3 dose levels of Poly E, the dose of 400 mg twice daily (total of 800 mg EGCG daily) may be preferable with a DLT rate of 6%.

Table 3. Adverse events by treatment group

	Poly E ($n = 30$)				Placebo ($n = 10$)				
Any toxicity, *n* (%)	**Grade I**	**Grade II**	**Grade III**	**Total, *n* (%)**	**Grade I**	**Grade II**	**Grade III**	**Total, *n* (%)**	***P*[a]**
Gastrointestinal									
Nausea	8	0	0	8 (27)	2	0	0	2 (20)	0.31
Diarrhea	2	1	0	3 (10)	1	1	0	2 (20)	0.17
Constipation	3	0	0	3 (10)	0	0	0	0	0.41
Indigestion	9	0	1[b]	10 (33)	2	0	0	2 (20)	0.20
Abdominal pain	1	0	0	1 (3)	2	0	0	2 (20)	0.14
Flatulence	0	1	0	1 (3)	1	0	0	1 (10)	0.19
Gastrointestinal bleed	0	0	1[b]	1 (3)	0	0	0	0	0.75
Weight gain	0	1[b]	0	1 (3)	0	0	0	0	0.75
Cardiopulmonary									
Palpitations	1	0	0	1 (3)	1	0	0	1 (10)	0.38
Dyspnea	0	0	0	0	1	0	0	1 (10)	0.25
Cough	0	0	0	0	1	0	0	1 (10)	0.25
Metabolic/Hematologic									
Transaminitis	2	0	1[b]	3 (10)	0	0	0	0	0.41
Hyperbilirubinemia	0	0	0	0	1	0	0	1 (10)	0.25
High alkaline phosphatase	2	0	0	2 (7)	0	0	0	0	0.56
High lipase	1	1	0	2 (7)	0	0	0	0	0.56
Hyperuricemia	1	0	0	1 (3)	1	0	0	1 (10)	0.38
Proteinuria	3	0	0	3 (10)	1	0	0	1 (10)	0.44
Anemia	2	0	0	2 (7)	0	0	0	0	0.56
Neurologic									
Headache	0	2	0	2 (7)	2	0	0	2 (20)	0.11
Confusion	0	0	0	0	1	0	0	1 (10)	0.25
Insomnia	3	0	1[b]	4 (13)	0	0	0	0	0.30
Endocrine									
Irregular menses	1	0	0	1 (3)	1	0	0	1 (10)	0.38
Hot flashes	0	1	0	1 (3)	0	0	0	0	0.75
Flushing	0	0	0	0	1	0	0	1 (10)	0.25
Vaginal symptoms	0	0	0	0	1	0	0	1 (10)	0.25

NOTE: Only toxicities that were possibly related to study drug with at least a 5% incidence rate are listed.
[a]Comparison using the Fisher exact test.
[b]Indicates a DLT.

This study highlights the importance of assessing long-term toxicity for any chemopreventive agent being developed for chronic use in healthy individuals. The toxicities that we observed with Poly E were consistent with the published literature. For example, we had a case of grade III rectal bleeding and liver function abnormalities. A 9-month study in Beagle dogs showed significant gastrointestinal toxicity and mortality when Poly E was administered in the fasting compared with the fed state (21). Therefore, Poly E was administered within 1 hour after a substantial meal in this trial to minimize gastrointestinal toxicities. A recent review reported 34 cases of hepatitis following consumption of green tea supplements used as weight loss products (22). In a phase I dose escalation trial of Poly E 400 to 2,000 mg twice daily in patients with chronic lymphocytic leukemia, 33% developed grade I transaminitis (23). Although the pervading public perception is that dietary supplements are generally safe, these toxicities need to be taken into account when weighing the risk:benefit ratio of any chemopreventive agent.

Because individual dietary components have not been successful in preventing cancer (24–28), perhaps using a polyphenolic mixture may be more effective. In a mouse model of lung carcinogenesis, the mixture of catechins with Poly E had more antitumor activity than EGCG alone (29). Bioavailability is another common issue with dietary supplements and often the doses used in preclinical studies are not always achievable in humans. Previous studies have found low bioavailability of tea catechins (15, 30). However, when large pharmacologic doses of polyphenols are orally administered, peak plasma EGCG concentrations of 5 to 7 μmol/L are observed in humans, compared with 0.5 μmol/L with green tea consumption (11). We tested pharmacologic doses of Poly E (400–800 mg twice daily or a

Table 4. Secondary biomarker endpoints

	Poly E ($n = 26$)		Placebo ($n = 8$)		
Biomarker	**Baseline**	**6 mo**	**Baseline**	**6 mo**	***P*[a]**
Total urinary tea polyphenols, μmol/g Cr					
Mean (SD)	16.8 (29.2)	163.8 (176.8)	8.0 (7.0)	19.9 (18.6)	
Mean absolute change from baseline (SD)	152.1 (186.4)		11.8 (13.7)		0.001
Percent change from baseline	905		148		
P[b]	<0.001		0.05		
Estradiol, pg/mL					
Mean (SD)	19.4 (39.5)	6.3 (13.3)	20.0 (35.1)	3.4 (2.6)	
Mean absolute change from baseline (SD)	−13.6 (34.2)		−19.7 (37.7)		0.98
Percent change from baseline	−70.1		−98.5		
P[b]	0.05		0.18		
Testosterone, ng/dL					
Mean (SD)	18.9 (14.3)	16.9 (9.7)	22.0 (17.1)	17.5 (11.9)	
Mean absolute change from baseline (SD)	−2.3 (9.5)		−5.1 (8.6)		0.58
Percent change from baseline	−12.2		−23.2		
P[b]	0.29		0.14		
SHBG, nmol/L					
Mean (SD)	53.1 (22.2)	43.9 (18.0)	68.4 (40.1)	52.2 (14.9)	
Mean absolute change from baseline (SD)	−7.2 (15.9)		−16.5 (30.5)		0.81
Percent change from baseline	−13.5		−24.1		
P[b]	0.03		0.17		
IGF-1, ng/mL					
Mean (SD)	157.3 (48.1)	147.8 (43.2)	159.9 (45.0)	170.8 (35.1)	
Mean absolute change from baseline (SD)	0.3 (34.7)		2.7 (36.8)		0.89
Percent change from baseline	0.2		1.7		
P[b]	0.97		0.84		
IGFBP-3, ng/mL					
Mean (SD)	4,077 (1,200)	4,383 (1,003)	4,122 (947)	4,506 (1,257)	
Mean absolute change from baseline (SD)	462 (921)		250 (782)		0.91
Percent change from baseline	11.3		6.1		
P[b]	0.02		0.40		
IGF-1/IGFBP-3					
Mean (SD)	0.048 (0.056)	0.034 (0.008)	0.040 (0.011)	0.040 (0.012)	
Mean absolute change from baseline (SD)	−0.015 (0.062)		−0.001 (0.006)		0.25
Percent change from baseline	−31.3		−2.5		
P[b]	0.24		0.77		
Ki-67, %					
Mean (SD)	1.5 (1.4)	2.5 (2.8)	0.8 (1.1)	1.3 (1.7)	
Mean absolute change from baseline (SD)	1.0 (3.1)		0.5 (2.4)		0.76
Percent change from baseline	66.7		62.5		
P[b]	0.19		0.58		
ER, %					
Mean (SD)	32.0 (17.6)	27.6 (14.8)	26.2 (16.2)	15.9 (14.4)	
Mean absolute change from baseline (SD)	−4.4 (14.9)		−10.2 (21.4)		0.76
Percent change from baseline	−13.8		−38.9		
P[b]	0.23		0.25		
MD, %					
Mean (SD)	16.4 (15.6)	17.1 (16.1)	15.5 (10.4)	16.6 (12.1)	
Mean absolute change from baseline (SD)	0.7 (5.6)		1.1 (4.0)		0.73
Percent change from baseline	4.3		7.1		
P[b]	0.58		0.46		

Abbreviation: MD, mammographic density.

[a]Comparing the absolute change from baseline in the Poly E versus placebo groups using Wilcoxon rank-sum test.

[b]Comparing follow-up to baseline by paired *t* test.

total of 800–1,600 mg of EGCG daily) with the EGCG content equivalent to 8 to 24 cups of brewed green tea daily. We showed high levels of urinary EGC and related metabolites (>150 μmol/g Cr) at these doses, compared with individuals who drink upward of 4 to 5 cups of green tea daily with urinary metabolites in the 50 to 100 μmol/g Cr range (18).

For the secondary exploratory analyses, we focused on systemic biomarkers that have been correlated with breast cancer risk, such as circulating sex steroid hormones (31) and IGF axis markers (32). Observational studies have correlated these biomarkers of breast cancer risk with green tea intake. In a cross-sectional study from Japan, higher serum IGF-1 levels, which have been hypothesized to promote rather than prevent cancer growth, were positively associated with green tea consumption (33). Green tea intake has also been correlated with lower circulating estrogen levels in pre- and postmenopausal women (34, 35). Proposed mechanisms include tea polyphenols that prevent binding of estrogen to its receptor in breast cancer cells (36) and inhibition of aromatase activity (37, 38). In our trial, the Poly E intervention resulted in favorable but not statistically significant changes in serum estradiol and IGF-1/IGFBP-3 ratio. Because we did not adjust for multiple comparisons, we should interpret these trends in biomarker changes with caution given the small sample size. Our results are consistent with a recently published trial of 103 postmenopausal women randomized to a 2-month intervention of placebo versus Poly E 400 or 800 mg daily (39). Administration of Poly E did not produce consistent patterns of changes in estradiol, testosterone, SHBG, IGF-1, and IGFBP-3. Other explanations for the negative biomarker results include the relatively short-term drug intervention or Poly E mediating its effects via alternative pathways.

The most well-documented modifiable biomarkers of breast cancer risk include mammographic density (40), Ki-67 (41), and ER (42) expression in benign breast tissue. One study showed that daily green tea drinkers had significantly lower percentage of mammographic density (19.5%) than non-tea drinkers (21.7%, $P = 0.002$; ref. 43). We did not observe a significant change in breast density or the Ki-67 proliferation index after 6 months of Poly E. Of note, the yield of epithelial cells from the random core biopsies and low baseline levels of Ki-67 staining in benign breast tissue may have limited our ability to detect change over time with this drug intervention. In addition, the 1-month washout period for tea consumption may have been insufficient to change baseline breast density measurements among regular green tea drinkers. ER expression in benign breast epithelium increases with age, postmenopausal status, and increasing morphologic abnormality, supporting a positive correlation with breast cancer risk (42, 44, 45). We observed a nonsignificant decrease in mean ER-α expression in the Poly E and placebo groups. We did not measure catechin levels at the tissue level and a potential reason for these negative results may be due to low achievable tissue concentrations of these polyphenols. A recent trial of Poly E 800 mg daily for 3 to 6 weeks in patients with prostate cancer showed low bioaccumulation of green tea polyphenols in prostate tissue (46). Therefore, bioavailability at the tissue level may have influenced the effects of Poly E on breast tissue–based biomarkers.

Bioavailability of green tea is also influenced by host-related factors, such as genetic polymorphisms which modulate the metabolism of tea polyphenols. A single G to A transition at codon 158 of *COMT* results in an amino acid change causing a 3- to 4-fold decrease in enzymatic activity (47). Data from the Shanghai Cohort Study showed that individuals who were homozygous for the low-activity associated *COMT* genotype (*AA*) had significantly lower urinary levels of tea polyphenol metabolites relative to those who had at least one high-activity allele (18). We did not observe a significant association between *COMT* genotype and urinary tea polyphenols among Poly E–treated women. However, our sample size was likely too small to show an association given the 18% incidence of the low-activity (*AA*) genotype.

We showed the feasibility of enrolling breast cancer survivors in an early-phase chemoprevention trial with frequent study visits and involving invasive procedures (e.g., core breast biopsy). Secondary prevention trials in breast cancer survivors evaluating the contralateral breast for surrogate endpoint biomarkers are a useful clinical model for testing novel chemopreventive agents (48). These women have a risk of developing contralateral breast primaries of 0.5% to 1% per year (49). One study showed a high concordance of 70% among women diagnosed with an ER-negative primary breast cancer having an ER-negative contralateral breast cancer (50). Therefore, this serves as a relevant clinical model for testing chemopreventive agents targeting ER-negative breast cancer.

Strengths of this study include the novel adaptive study design for assessing long-term toxicity of a potential chemopreventive agent. The placebo group provided the background rate of lower grade toxicities, as well as important reference levels for all biomarkers. We had relatively good participant retention with 85% completing the 6-month evaluations. The main weakness is the relatively small sample size for assessing secondary biomarker endpoints. Future studies using this clinical model will need to account for rates of missing data due to inadequate samples for biomarker analysis, particularly for the tissue biomarkers. Our goal was to obtain preliminary data on the biologic effects of Poly E, which may elucidate potential mechanisms of action that would inform future clinical efficacy trials. Ongoing trials of green tea in breast cancer include a study of 50 women with newly diagnosed ductal carcinoma *in situ* given Poly E 600 mg daily for 4 to 6 weeks before surgical resection (http://clinicaltrials.gov, NCT01060345) and a placebo-controlled trial of a 1-year intervention of a green tea extract (800 mg EGCG daily) in postmenopausal women with high mammographic density (NCT00917735). These trials include biomarker endpoints, such as Ki-67 and mammographic density.

In conclusion, using a novel clinical trial design for phase I testing that evaluates long-term toxicity, we determined

the MTD for Polyphenon E to be 600 mg twice daily (total of 1,200 mg EGCG daily), which will serve as the upper safety limit in future long-term intervention trials. We have also shown the bioavailability of Poly E at pharmacologic levels and the feasibility of conducting an early-phase chemoprevention trial in a highly motivated group of women with HR-negative breast cancer. However, to conduct more efficient chemoprevention studies, we need to validate surrogate endpoint biomarkers for short-term breast cancer risk assessment. In general, the public perception is that dietary supplements are safe and, therefore, may gain wider acceptance in the prevention setting compared with pharmacologic drugs. These agents need to be rigorously tested and future studies should evaluate the clinical efficacy of Poly E on biomarkers of breast cancer risk.

Disclosure of Potential Conflicts of Interest

P. Brown is a Consultant/Advisory Board member of Susan G. Komen for the Cure. No potential conflicts of interest were disclosed by the other authors.

The Editor-in-Chief of *Cancer Prevention Research* is an author of this article. In keeping with the AACR's Editorial Policy, the paper was peer reviewed and a member of the AACR's Publications Commitee rendered the decision concerning acceptability.

Authors' Contributions

Conception and design: K.D. Crew, P. Brown, H. Greenlee, J. Chang, D.L. Hershman

Development of methodology: K.D. Crew, H. Greenlee, B. Arun, J. Chang, H. Hibshoosh, D.L. Hershman

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.D. Crew, T.B. Bevers, B. Arun, C. Hudis, H.L. McArthur, J. Chang, M. Rimawi, H. Hibshoosh, M.B. Terry, R.M. Santella, D. L. Hershman

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.D. Crew, P. Brown, B. Arun, C. Hudis, A. Wang, H. Hibshoosh, M.B. Terry, R.M. Santella, D.L. Hershman

Writing, review, and/or revision of the manuscript: K.D. Crew, P. Brown, H. Greenlee, T.B. Bevers, B. Arun, C. Hudis, H.L. McArthur, J. Chang, M. Rimawi, L. Vornik, T.L. Cornelison, A. Wang, H. Hibshoosh, M.B. Terry, R.M. Santella, S.M. Lippman, D.L. Hershman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.B. Bevers, C. Hudis, H.L. McArthur, L. Vornik, H. Hibshoosh, D.L. Hershman

Study supervision: K.D. Crew, T.B. Bevers, L. Vornik, T.L. Cornelison, D.L. Hershman

Protocol administration: L. Vornik

Pathologist and reviewed the biopsy slides related to this study: A. Ahmed

Grant Support

The study was supported by the National Cancer Institute (grant N01-CN-35159), the National Institute of Environmental Health Sciences (grant ES009089), and the American Cancer Society (grant ACS MRSG-08-021-01-CNE).

Clinical Trial Registration: clinicaltrials.gov identifier: NCT00516243.

Received March 16, 2012; revised May 25, 2012; accepted June 13, 2012; published OnlineFirst July 24, 2012.

References

1. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371–88.
2. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA 2006;295:2727–41.
3. Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, et al. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 2011;364:2381–91.
4. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
5. Ogunleye AA, Xue F, Michels KB. Green tea consumption and breast cancer risk or recurrence: a meta-analysis. Breast Cancer Res Treat 2010;119:477–84.
6. Nakachi K, Suemasu K, Suga K, Takeo T, Imai K, Higashi Y. Influence of drinking green tea on breast cancer malignancy among Japanese patients. Jpn J Cancer Res 1998;89:254–61.
7. Inoue M, Tajima K, Mizutani M, Iwata H, Iwase T, Miura S, et al. Regular consumption of green tea and the risk of breast cancer recurrence: follow-up study from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), Japan. Cancer Lett 2001;167:175–82.
8. Wu AH, Yu MC, Tseng CC, Hankin J, Pike MC. Green tea and risk of breast cancer in Asian Americans. Int J Cancer 2003;106:574–9.
9. Wu AH, Tseng CC, Van Den Berg D, Yu MC. Tea intake, COMT genotype, and breast cancer in Asian-American women. Cancer Res 2003;63:7526–9.
10. Yang CS. Inhibition of carcinogenesis by tea. Nature 1997;389:134–5.
11. Yang CS, Wang X, Lu G, Picinich SC. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 2009;9:429–39.
12. Stuart EC, Scandlyn MJ, Rosengren RJ. Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer. Life Sci 2006;79:2329–36.
13. Chang PY, Mirsalis J, Riccio ES, Bakke JP, Lee PS, Shimon J, et al. Genotoxicity and toxicity of the potential cancer-preventive agent polyphenon E. Environ Mol Mutagen 2003;41:43–54.
14. Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001;10:53–8.
15. Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003;9:3312–9.
16. Cheung YK, Chappell R. Sequential designs for phase I clinical trials with late-onset toxicities. Biometrics 2000;56:1177–82.
17. Byng JW, Yaffe MJ, Lockwood GA, Little LE, Tritchler DL, Boyd NF. Automated analysis of mammographic densities and breast carcinoma risk. Cancer 1997;80:66–74.
18. Inoue-Choi M, Yuan JM, Yang CS, Van Den Berg DJ, Lee MJ, Gao YT, et al. Genetic association between the COMT genotype and urinary levels of tea polyphenols and their metabolites among daily green tea drinkers. Int J Mol Epidemiol Genet 2010;1:114–23.
19. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83.
20. Cheung YK, Chappell R. A simple technique to evaluate model sensitivity in the continual reassessment method. Biometrics 2002;58:671–4.
21. Kapetanovic IM, Crowell JA, Krishnaraj R, Zakharov A, Lindeblad M, Lyubimov A. Exposure and toxicity of green tea polyphenols in fasted and non-fasted dogs. Toxicology 2009;260:28–36.
22. Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C, et al. Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 2009;65:331–41.
23. Shanafelt TD, Call TG, Zent CS, LaPlant B, Bowen DA, Roos M, et al. Phase I trial of daily oral Polyphenon E in patients with asymptomatic Rai stage 0 to II chronic lymphocytic leukemia. J Clin Oncol 2009;27:3808–14.

24. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 1994;330:1029–35.
25. Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, et al. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J Natl Cancer Inst 1996;88:1560–70.
26. Omenn GS, Goodman G, Thornquist M, Grizzle J, Rosenstock L, Barnhart S, et al. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res 1994;54 Suppl:2038s–43s.
27. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297:2351–9.
28. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2011;306:1549–56.
29. Fu H, He J, Mei F, Zhang Q, Hara Y, Ryota S, et al. Lung cancer inhibitory effect of epigallocatechin-3-gallate is dependent on its presence in a complex mixture (polyphenon E). Cancer Prev Res 2009;2:531–7.
30. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002;11:1025–32.
31. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group. Ann Intern Med 1999;130:270–7.
32. Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010;11:530–42.
33. Maruyama K, Iso H, Ito Y, Watanabe Y, Inaba Y, Tajima K, et al. Associations of food and nutrient intakes with serum IGF-I, IGF-II, IGFBP-3, TGF-b1, total SOD activity and sFas levels among middle-aged Japanese: the Japan Collaborative Cohort study. Asian Pac J Cancer Prev 2009;10 Suppl:7–22.
34. Nagata C, Kabuto M, Shimizu H. Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women. Nutr Cancer 1998;30:21–4.
35. Wu AH, Arakawa K, Stanczyk FZ, Van Den Berg D, Koh WP, Yu MC. Tea and circulating estrogen levels in postmenopausal Chinese women in Singapore. Carcinogenesis 2005;26:976–80.
36. Farabegoli F, Barbi C, Lambertini E, Piva R. (-)-Epigallocatechin-3-gallate downregulates estrogen receptor alpha function in MCF-7 breast carcinoma cells. Cancer Detect Prev 2007;31:499–504.
37. Satoh K, Sakamoto Y, Ogata A, Nagai F, Mikuriya H, Numazawa M, et al. Inhibition of aromatase activity by green tea extract catechins and their endocrinological effects of oral administration in rats. Food Chem Toxicol 2002;40:925–33.
38. Goodin MG, Rosengren RJ. Epigallocatechin gallate modulates CYP450 isoforms in the female Swiss-Webster mouse. Toxicol Sci 2003;76:262–70.
39. Wu AH, Spicer D, Stanczyk FZ, Tseng CC, Yang CS, Pike MC. Effect of 2-month controlled green tea intervention on lipoprotein cholesterol, glucose, and hormone levels in healthy postmenopausal women. Cancer Prev Res 2012;5:393–402.
40. Cuzick J, Warwick J, Pinney E, Duffy SW, Cawthorn S, Howell A, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. J Natl Cancer Inst 2011;103:744–52.
41. Khan QJ, Kimler BF, Clark J, Metheny T, Zalles CM, Fabian CJ. Ki-67 expression in benign breast ductal cells obtained by random periareolar fine needle aspiration. Cancer Epidemiol Biomarkers Prev 2005;14:786–9.
42. Khan SA, Rogers MA, Khurana KK, Meguid MM, Numann PJ. Estrogen receptor expression in benign breast epithelium and breast cancer risk. J Natl Cancer Inst 1998;90:37–42.
43. Wu AH, Ursin G, Koh WP, Wang R, Yuan JM, Khoo KS, et al. Green tea, soy, and mammographic density in Singapore Chinese women. Cancer Epidemiol Biomarkers Prev 2008;17:3358–65.
44. Shoker BS, Jarvis C, Sibson DR, Walker C, Sloane JP. Oestrogen receptor expression in the normal and pre-cancerous breast. J Pathol 1999;188:237–44.
45. Shaaban AM, Sloane JP, West CR, Foster CS. Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor-alpha and Ki-67 expression. Am J Pathol 2002;160:597–604.
46. Nguyen MM, Ahmann FR, Nagle RB, Hsu CH, Tangrea JA, Parnes HL, et al. Randomized, double-blind, placebo controlled trial of polyphenon E in prostate cancer patients before prostatectomy: evaluation of potential chemopreventive activities. Cancer Prev Res 2012;5:290–8
47. Dawling S, Roodi N, Mernaugh RL, Wang X, Parl FF. Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. Cancer Res 2001;61:6716–22.
48. Stearns V, Gallagher A, Kleer CG, Singh B, Freedman M, Haddad BR, et al. A pilot study to establish a clinical model to perform phase II studies of breast cancer chemopreventive agents in women at high risk with biomarkers as surrogate endpoints for activity. Clin Cancer Res 2004;10:8332–40.
49. Malone KE, Begg CB, Haile RW, Borg A, Concannon P, Tellhed L, et al. Population-based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or BRCA2. J Clin Oncol 2010;28:2404–10.
50. Swain SM, Wilson JW, Mamounas EP, Bryant J, Wickerham DL, Fisher B, et al. Estrogen receptor status of primary breast cancer is predictive of estrogen receptor status of contralateral breast cancer. J Natl Cancer Inst 2004;96:516–23.