Randomized Phase II Trial of Polyphenon E versus Placebo in Patients at High Risk of Recurrent Colonic Neoplasia

Frank A. Sinicrope[1], Thomas R. Viggiano[1], Navtej S. Buttar[1], Louis M. Wong Kee Song[1], Kenneth W. Schroeder[1], Robert E. Kraichely[1], Mark V. Larson[1], Robert E. Sedlack[1], John B. Kisiel[1], Christopher J. Gostout[1], Abdul M. Kalaiger[1], Árpád V. Patai[1,6], Gary Della'Zanna[2], Asad Umar[2], Paul J. Limburg[1], Jeffrey P. Meyers[3], Nathan R. Foster[3], Chung S. Yang[4], and Stephen Sontag[5]

ABSTRACT

Polyphenon E (Poly E) is a green tea polyphenol preparation whose most active component is epigallocatechin gallate (EGCG). We studied the cancer preventive efficacy and safety of Poly E in subjects with rectal aberrant crypt foci (ACF), which represent putative precursors of colorectal cancers. Eligible subjects had prior colorectal advanced adenomas or cancers, and had ≥5 rectal ACF at a preregistration chromoendoscopy. Subjects ($N = 39$) were randomized to 6 months of oral Poly E (780 mg EGCG) daily or placebo. Baseline characteristics were similar by treatment arm (all P >0.41); 32 of 39 (82%) subjects completed 6 months of treatment. The primary endpoint was percent reduction in rectal ACF at chromoendoscopy comparing before and after treatment. Among 32 subjects (15 Poly E, 17 placebo), percent change in rectal ACF number (baseline vs. 6 months) did not differ significantly between study arms (3.7% difference of means; $P = 0.28$); total ACF burden was also similar (−2.3% difference of means; $P = 0.83$). Adenoma recurrence rates at 6 months were similar by arm ($P > 0.35$). Total drug received did not differ significantly by study arm; 31 (79%) subjects received ≥70% of prescribed Poly E. Poly E was well tolerated and adverse events (AE) did not differ significantly by arm. One subject on placebo had two grade 3 AEs; one subject had grade 2 hepatic transaminase elevations attributed to treatment. In conclusion, Poly E for 6 months did not significantly reduce rectal ACF number relative to placebo. Poly E was well tolerated and without significant toxicity at the dose studied.

Prevention Relevance: We report a chemoprevention trial of polyphenon E in subjects at high risk of colorectal cancer. The results show that polyphenon E was well tolerated, but did not significantly reduce the number of rectal aberrant crypt foci, a surrogate endpoint biomarker of colorectal cancer.

Introduction

Colorectal cancer is the third most common cause of cancer-related death in the United States and ranks second as cause of cancer-related mortality (1). Pharmacologic strategies to prevent this malignancy remain a major unmet need. Green tea consumption as a beverage has been extensively studied for possible cancer preventive effects (2). Epidemiologic studies have suggested an association of green tea intake of at least 10 cups per day (3) with a reduced risk of colorectal cancer (4). In rodent models, dietary polyphenon E (Poly E) reduced the total number of colorectal aberrant crypt foci (ACF) and decreased the percentage of ACF with high-grade dysplasia in the azoxymethane (AOM) model of colon carcinogenesis (5–7). ACF are putative precursor lesions of colorectal cancers that can be detected using chromoendoscopy (8). To date, interventional studies evaluating green tea preparations for chemoprevention of colorectal cancer in humans are limited to two studies from Japan (3) and Korea (9) that support the further evaluation of Poly E for colon cancer prevention. Data also exist in subjects with established cancer of the prostate (10) and chronic lymphocytic leukemia (CLL; ref. 11) where clinical responses were observed.

Green tea constituents include polyphenols that have been shown to confer cancer preventive properties in animal model systems of colorectal cancer and other tumor types (12, 13). The major and most biologically active green tea polyphenol is

[1]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota. [2]Gastrointestinal and Other Cancers Research Group, Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland. [3]Department of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, Minnesota. [4]Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, The State University of New Jersey, Piscataway, New Jersey. [5]Section of Gastroenterology, Edward Hines, Jr. VA Hospital, Hines, Illinois. [6]Department of Internal Medicine and Hematology, Semmelweis University, Budapest, Hungary.

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

Corresponding Author: Frank A. Sinicrope, Mayo Clinic, 200 1st St SW, Rochester, MN 55902. Phone: 507-255-5713; Fax: 507-255-6318; E-mail: sinicrope.frank@mayo.edu

Cancer Prev Res 2021;14:573–80

doi: 10.1158/1940-6207.CAPR-20-0598

epigallocatechin-3-gallate (EGCG). Poly E is a defined green tea polyphenol preparation containing 55%–72% of the EGCG and other catechins (∼25%) with minimal amounts of caffeine (<1.0%), theobromine (<1.0%) and gallic acid (<0.5%; refs. 14, 15). Poly E and EGCG have been formulated into capsules by the Chemoprevention Agent Development Research Group at the NCI for prevention and treatment studies in a variety of cancers (11, 16).

Most colorectal cancer chemoprevention studies have utilized adenoma recurrence as the primary endpoint based upon evidence establishing adenomas as precursor lesions of colorectal cancers (17). However, these trials require large numbers of patients with extended clinical follow-up, and entail considerable expense. ACF were first described by Bird and colleagues (18) in the murine colon after exposure to the carcinogen, azoxymethane (AOM). ACF are the earliest morphologically detectable lesions in colorectal mucosa and are believed to be precursors of adenomas and cancers (19, 20) as shown by their increased prevalence, density, and frequency of dysplasia in subjects over the age of 40 years (19, 21). Furthermore, human ACF number is higher in the left versus right colon (21–23), which parallels the distribution of sporadic colonic neoplasms. Also, studies in humans have shown that ACF number and size are increased in patients with versus without prior or current colorectal neoplasms (19, 22, 24). While ACF are not visible using standard white-light colonoscopes, the use of chromoendoscopy with a mucosal dye, such as methylene blue, allows ACF to be readily identified and quantified (8, 21, 25). Preclinical studies support the utility of ACF as a surrogate endpoint biomarker (SEB) for chemoprevention trials. In this regard, ACF are used as an SEB for the efficacy of antitumor agents in the chemoprevention program of the NCI (26).

We conducted a phase II chemoprevention study to determine the effect of treatment with Poly E on rectal ACF in patients at high risk of colorectal neoplasia and also to examine its tolerability and safety profile.

Materials and Methods

Study population and design

We conducted a randomized, double-blinded, and placebo-controlled trial in adult subjects ($n = 39$) age 40 years or older with a history of current or prior advanced colorectal adenomas or cancer. Advanced adenomas are defined as adenomas ≥1 cm and/or with tubulovillous or villous histology, or high-grade dysplasia. A full colonoscopy was required within 45 days prior to randomization. If a colonoscopy was done within this time frame, then a flexible sigmoidoscopy with chromoendoscopy was performed in the preregistration phase to identify subjects with at least 5 rectal ACF. Otherwise, colonoscopy was performed per standard of care and chromoendoscopy of the sigmoid colon and rectum was added for ACF evaluation. Subjects with ≥ 5 rectal ACF who met all eligibility criteria were randomized to receive either placebo or Poly E. Patients were enrolled at Mayo Clinic (Rochester, MN) and Hines Veteran's Administration Hospital (Hines, IL). Patients were stratified on the basis of the Institution where subject randomization occurred, prior use of low dose aspirin (81 mg; yes vs. no), and disease history (advanced adenomas or prior colon cancer). Consumption of over-the-counter green tea or green tea extract ≤ 6 weeks prior to registration/randomization and/or during study treatment was prohibited. Participants were randomized in a blinded fashion to receive either Polyphenon E (Poly E; two 300-mg capsules orally twice daily containing ∼65% EGCG, 25% other catechins and less than 0.6% caffeine; Arm 1) or matched placebo (Arm 2) in a 1:1 ratio using the Pocock–Simon dynamic allocation procedure (27). Poly E capsules were supplied and distributed under contract with Mitsui Norin via Aptuit, Inc. The study drugs were initiated within 7 days of randomization and taken on a continuous schedule for 6 consecutive months. Accrual was terminated at 39 subjects due to pending expiration of Poly E with need for repeat drug stability testing to ensure that the quality of the drug is maintained.

Chromoendoscopy was performed at surveillance colonoscopy or during a left-sided examination at flexible sigmoidoscopy if the subject had received prior colonoscopy within 90 days. ACF were identified at chromoendoscopy as crypts that stain darkly with methylene blue (compared with normal crypts) and had larger diameters with oval or slit-like lumens and also with thicker epithelial linings (19, 20). All eligible patients were randomized with documentation of baseline history, physical examination, ECOG performance status, and use of any concomitant medications. Blood samples were collected to ensure normal baseline values (chemistry panel, hematology panel, and liver function tests). Participants with fewer than 5 rectal ACFs at baseline were considered ineligible and all ACF were biopsied and biobanked. At preregistration endoscopy, all polyps ≥ 2 mm were removed and submitted for pathologic evaluation. Adenoma recurrence was determined based on the recurrence of adenomatous polyps at the 6-month end-of-treatment flexible sigmoidoscopy and up to 5 years follow-up postrandomization.

Post-randomization safety evaluations were performed every month via telephone interview to monitor compliance with the study medications, use of concomitant medications, and to identify and record adverse events along with monthly blood collection for assessment of liver function. A follow-up clinic visit was conducted at month 6, which included a physical examination and laboratory testing. Safety and tolerability of the study drugs was evaluated by reviewing patterns of adverse events, within and across the intervention and placebo group, via frequency tables and univariate statistics. The NCI CTC Version 3.0 was used to grade all adverse events. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) reviewed accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office.

All enrolled patients had a colonoscopy or flexible sigmoidoscopy combined with chromoendoscopy of the sigmoid colon and rectum at baseline and at end of treatment (6 months postrandomization) with ACF assessment. At baseline, a cluster of ≥ 5 ACF were identified in the rectum with placement of a mucosal tattoo (India ink) to facilitate identification of this same region after 6 months on study. At end of treatment, the tattooed region was reexamined and ACF were quantified after 6 months of continuous drug therapy. At baseline chromoendoscopy, biopsy of any sigmoid but not rectal ACF was performed. At the 6-month exam, up to 10 sigmoid ACF and up to 10 rectal ACF including the 5 index ACF were biopsied. The biopsied ACF tissues were then biobanked. All study procedures were performed by a limited number of endoscopists at Mayo Clinic (Rochester, MN) and by Dr. Stephen Sontag at Hines, VA. Written informed consent was obtained for all study subjects. The study was approved by the Institutional Review Board at both Institutions. The studies were conducted in accordance with the Declaration of Helsinki.

Study procedures

Details of the chromoendoscopy procedure for evaluation of rectal ACF are as follows. The rectum was defined based upon measurement of the distance from the anal verge to the middle rectal fold (∼15 cm). After inspection and removal of any polyps, the endoscope was withdrawn into the rectum and 60 cc of 10% Mucomyst solution was applied with a spray catheter to coat the mucosa beginning at 25 cm from anal verge (dwell time of 1–2 minutes). After washing the mucosa with 60 mL of water to remove residual mucus, the rectal mucosa was then painted with methylene blue dye (0.2%) in a circumferential fashion using a spray catheter beginning at 25 cm from anal verge (dwell time of 2 minutes). Assessment of rectal ACF was then performed by slow, deliberate pull-back of the endoscope. A mucosal tattoo was placed at the site of an index cluster of ≥ 5 rectal ACF. Still photography of regions of interest were performed and the procedure was videotaped to enable future review. Total ACF number and distance from the anal verge were recorded. Among ACF characteristics that were recorded on case report forms were absolute number of ACF, distance from anal verge, and ACF size in mm.

Statistical considerations

The primary study endpoint was to determine the association of Poly E treatment with percent change in the number of rectal ACF (% change in ACF) identified during the baseline and end of treatment chromoendoscopy exams. On the basis of a prior chemoprevention trial in subjects at high risk of colorectal cancer completed by the Mayo Clinic Cancer Prevention Network (28), we designed our trial with 80% power to observe at least a 40% decrease in the number of rectal ACF at 6 months from baseline to consider that Poly E showed a significant cancer preventive effect in our study population (28). The CPN trial found that approximately 60% of preregistered cases had ≥ 5 rectal ACF and that the percent change in rectal ACF was bidirectional (i.e., increased and decreased; ref. 28). Data from this trial supported our conservative estimate for the SD for the distribution of percent change in rectal ACF to be in the range of possible values (i.e., −1 to 1, which is 2) divided by 4 (i.e., 0.5). Given these considerations, our plan was to test the hypothesis of no change in the number of rectal ACF at 6 months (i.e., Ho) versus at least a 40% decrease in the number of rectal ACF at 6 months (i.e., Ha). Here, we assumed that the % decrease in ACF is normally distributed with equal dispersion (i.e., variance) among the placebo and Poly E arms. Assuming equal observed standard deviations (50%) across the treatment and placebo arms, a sample size of 21 participants per arm yields 80% power (one-sided, two-sample t test, alpha = 0.05) to detect a decrease of at least 40%, or 80% of a SD, in percent change in rectal ACF between the active agent group versus the placebo group. To achieve the goal of 21 eligible per arm (42 total) that would complete both baseline and 6-month evaluations, our goal was to randomize 50 total patients (25 per arm). Study accrual occurred over an 18-month period and was suspended early due to a pending expiration of the supply of Poly E. For our primary endpoint, descriptive statistics and box plots are reported. Because of skewness and outliers, the Wilcoxon rank-sum test was used for comparing the percent change in rectal ACF between the arms.

Secondary study endpoints included a comparison of baseline demographic and clinical data, treatment tolerability, and adverse events. Only patients who received > 70% of total expected dose of Poly E were included in the evaluation of the total ACF burden (sum of ACF size in mm across aggregate ACFs), and the adenoma recurrence rate. For these endpoints, the Wilcoxon rank-sum test was used to compare continuous data by arm and the χ^2 test to compare categorical data. Descriptive statistics [median, range, mean, SD, frequency (%)] are shown in Results/Tables. Other than the primary endpoint (one-sided), other P values are reported as two-sided, where P values < 0.05 are considered statistically significant. Analyses were performed using SAS version 9.4 (SAS Institute Inc.).

Results

We screened 78 subjects with prior advanced adenomas ($n = 77$) or carcinoma ($n = 1$) and identified 39 (50%) subjects who had 5 or more rectal ACF at baseline chromoendoscopy and also met all other eligibility criteria for participation (**Fig. 1**). We randomized 39 total subjects to the treatment arm with Poly E ($n = 19$) or to the placebo arm ($n = 20$). Baseline characteristics of the study population were similar between treatment arms (all $P > 0.41$; **Table 1**). The median patient age was 62 years, 64% were male, 85% were white, 95% had documented prior advanced adenomas, and 72% denied chronic use of low dose aspirin (defined as 7 consecutive days for >3 weeks).

Of the 39 randomized patients, 32 (82%) had both baseline and 6-month ACF evaluations (7 discontinued treatment early in that 6 refused further treatment and 1 withdrew due to an

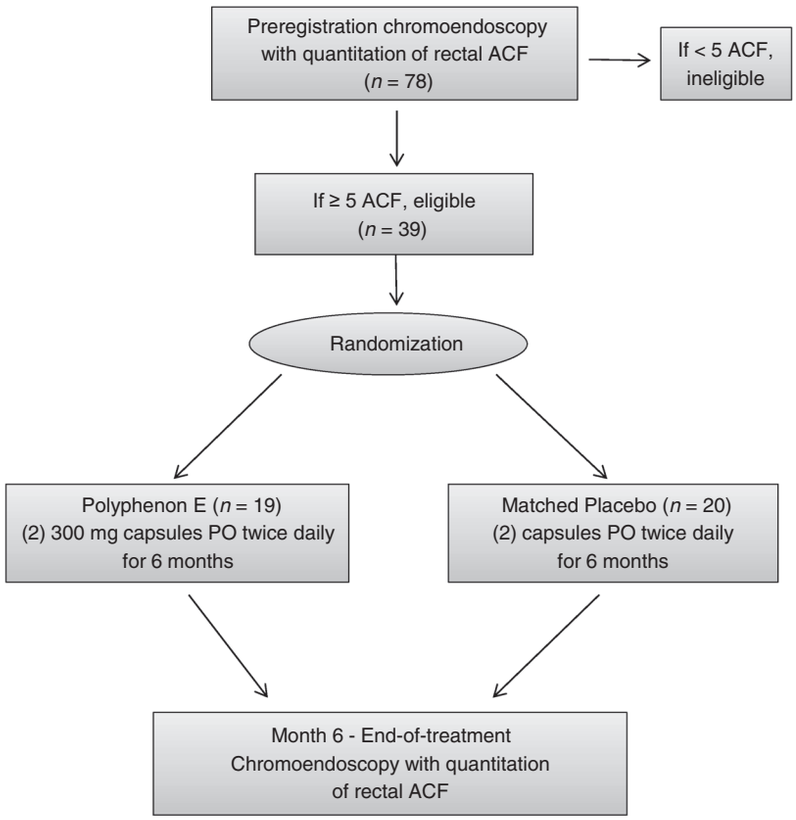

Figure 1.
Study schema for a randomized trial of Poly E versus placebo in patients with prior advanced colorectal adenomas or colon cancer.

Table 1. Clinical variables by study arm.

	Poly E ($n = 19$)	Placebo ($n = 20$)	Total ($n = 39$)	P
Age				0.87[a]
N	19	20	39	
Mean (SD)	62.1 (9.4)	61.4 (7.9)	61.7 (8.6)	
Median	63.0	62.0	62.0	
Q1–Q3	54.0–70.0	56.5–66.0	55.0–67.0	
Range	(46.0–77.0)	(48.0–77.0)	(46.0–77.0)	
Sex				0.58[b]
Female	6 (31.6%)	8 (40.0%)	14 (35.9%)	
Male	13 (68.4%)	12 (60.0%)	25 (64.1%)	
Race				0.41[b]
White	17 (89.55%)	16 (80.0%)	33 (84.6%)	
African American	2 (10.55%)	4 (20.0%)	6 (15.4%)	
Prior Aspirin (81 mg)				0.80[b]
No	14 (73.7%)	14 (70.0%)	28 (71.8%)	
Yes	5 (26.3%)	6 (30.0%)	11 (28.2%)	
Institution				0.89[b]
Hines Veterans admin.	8 (42.1%)	8 (40.0%)	16 (41.0%)	
Mayo Clinic	11 (57.9%)	12 (60.0%)	23 (59.0%)	
Disease history				0.97[b]
Advanced adenomas	18 (94.7%)	19 (95.0%)	37 (94.9%)	
Prior colon cancer	1 (5.3%)	1 (5.0%)	2 (5.1%)	

[a]Wilcoxon rank-sum test.
[b]χ^2 test.

adverse event). Evaluation of rectal ACF was performed using chromoendoscopy (**Fig. 2**). Of the 32 evaluable patients (15 Poly E; 17 Placebo), there was no significant differences in the primary endpoint of percent change in rectal ACF number comparing baseline to 6 months for each study arm (Poly E vs. placebo, 3.7% difference of mean values; one-sided $P = 0.28$; **Table 2**; **Fig. 3A**). Specifically, the placebo arm had a mean ACF change of 0% compared with a mean increase of 3.7% for the Poly E–treated patients. Furthermore, the total ACF burden (defined as the sum of the diameters of aggregated rectal ACF) was also similar between study arms (Poly E vs. placebo, −2.3% difference of mean values; $P = 0.83$, **Table 2**; **Fig. 3B**). Most patients received at least 70% of the prescribed treatment doses [31 (79%)], and 32 of the 39 patients (82%) received all 6 months of treatment. Similar results were found after excluding patients who received < 70% of Poly E treatment.

At the 6-month end of treatment evaluation, adenoma recurrence rates did not differ significantly among the study arms. Specifically, Poly E–treated patients had an adenoma recurrence rate of 29% compared with a recurrence rate of 35% for placebo patients ($P = 0.69$; **Table 2**). When only polyps ≥ 5 mm were considered, the adenoma recurrence rate decreased to 0% for the Poly E–treated patients compared with 6%

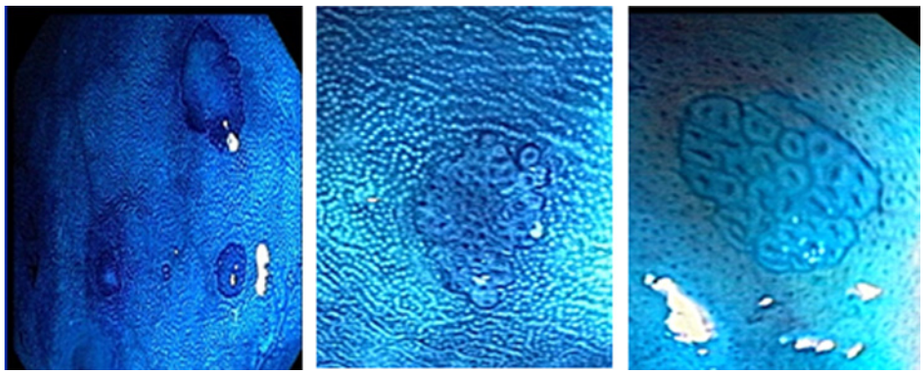

Figure 2.
ACF seen in the rectum at chromoendoscopy with use of methylene blue.

for the placebo-treated patients ($P = 0.36$). None of the patients developed an advanced adenoma during study treatment.

Treatment was well tolerated overall. Reasons for discontinuing treatment early included refusal of further treatment ($n = 6$) or an adverse event ($n = 1$). Subjects on the treatment arm had similar pill intake, estimated by pill counts and medication diaries, as those on the placebo arm ($P = 0.14$; **Table 2**). Adverse events (AE) did not differ significantly by study arm, and only one patient had any grade 3 or higher AE in the placebo arm; **Table 3**). This same patient had both grade 3 abdominal pain and grade 3 dyspepsia. One subject in the Poly E arm had grade 2 AEs consisting of hepatic transaminase elevations (**Table 3**). Holding Poly E resulted in normalization of transaminase values indicating a relationship to treatment.

Table 2. Results by study arm.

	Poly E ($n = 19$)	Placebo ($n = 20$)	Total ($n = 39$)	*P*
Baseline ACF number				0.38[a]
N	19	20	39	
Mean (SD)	12.2 (6.8)	11.5 (10.6)	11.8 (8.8)	
Median	9.0	8.0	9.0	
Range	(5.0–28.0)	(5.0–52.0)	(5.0–52.0)	
6-month ACF number				0.25[a]
N	15	17	32	
Mean (SD)	12.7 (10.01)	11.5 (12.3)	12.1 (11.2)	
Median	10.0	8.0	9.0	
Range	(2.0–44.0)	(2.0–53.0)	(2.0–53.0)	
ACF number percent change				0.28[a,b]
N	15	17	32	
Mean (SD)	3.7 (49.1)	0 (52.7)	1.7 (55.9)	
Median	0.0	0.0	0.0	
ACF size percent change				0.83[a]
N	15	16	31	
Mean (SD)	10.3 (61.1)	12.6 (102.8)	11.5 (83.9)	
Median	0.0	0.0	0.0	
Adenoma recurrence				0.69[c]
Missing	5	3	8	
No	10 (71.4%)	11 (64.75%)	21 (67.7%)	
Yes	4 (28.6%)	6 (35.3%)	10 (32.3%)	
Adenoma recurrence (≥5 mm)				0.36[c]
Missing	5	3	8	
No	14 (100.0%)	16 (94.1%)	30 (96.8%)	
Yes	0 (0.0%)	1 (5.9%)	1 (3.2%)	
Percent total dose received				0.14[a]
N	19	20	39	
Mean (SD)	83.2 (29.3)	91.7 (24.0)	87.6 (26.7)	

[a]Wilcoxon rank-sum test.
[b]One-sided *P* value.
[c]χ^2 test.

Colonoscopy and related pathology reports were collected on participants post study enrollment. Median follow-up time on the Poly E and placebo study arms was 39.1 and 36.4 months, respectively ($P = 0.44$). Similar numbers of post treatment colonoscopies were performed by study arm ($P = 0.75$). Among patients with available post study data ($N = 17$), recurrent adenomas were found at colonoscopy in 1 of 7 (14.3%) patients treated with Poly E versus 4/10 (40%) on the placebo arm ($P = 0.056$; Supplementary Table S1). Furthermore, the median time-to-adenoma recurrence post-treatment was delayed in the Poly E study arm to 62.7 months compared with 46.6 months the placebo arm.

Discussion

We conducted a phase II randomized and placebo-controlled trial of Poly E treatment in patients at high risk of metachronous colorectal neoplasia. Our high risk population, that is, prior advanced colorectal adenomas or colon cancer, differs from most other chemoprevention studies which have limited the study population to patients at average risk of colorectal cancer. We measured ACF regression as a surrogate endpoint biomarker (SEB) for cancer preventive efficacy based on the demonstrated efficacy of Poly E to reduce the total number of colorectal ACF in a rodent model of colon carcinogenesis (6), and data supporting ACF as precursors of colorectal cancer in humans (19–21). Evaluation of ACF as an SEB also enables one to measure short-term pharmacologic intervention on ACF regression which can facilitate human chemoprevention trials by making them of shorter duration, requiring fewer participants, and at lower cost.

The primary study endpoint of percent change in the number of rectal ACF between the baseline and end-of-

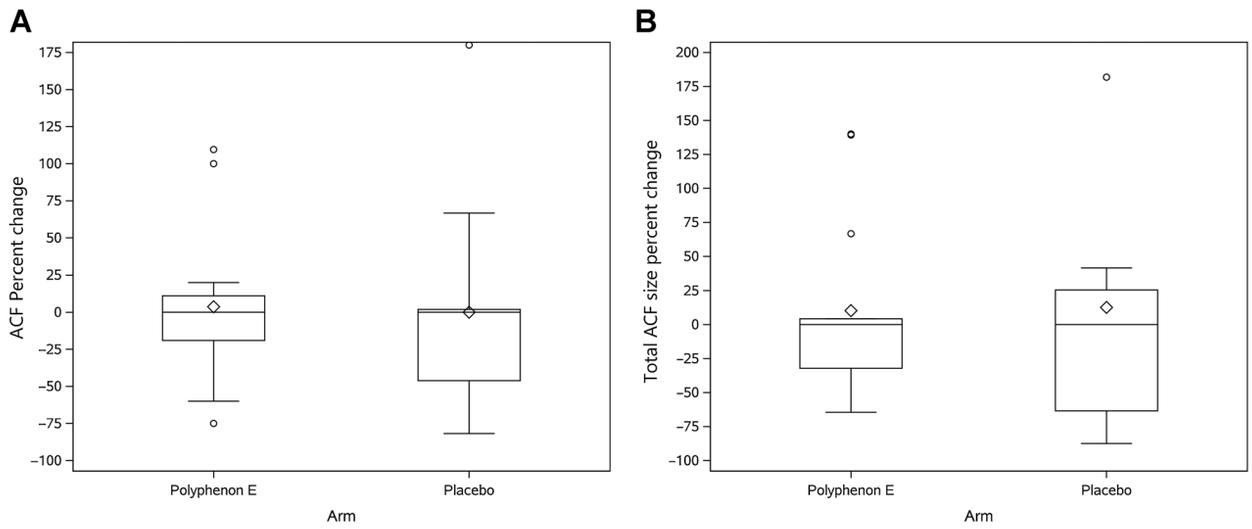

Figure 3.

A, Percent change in rectal ACF number comparing baseline and 6-month evaluations at chromoendoscopy (primary study endpoint). **B,** Percent change in aggregate ACF size (baseline vs. 6 months).

treatment exams was based on a modified intent-to-treat procedure, which was inclusive of all patients with baseline and 6-month ACF data. The requirement for ≥ 5 rectal ACF is per established precedent from at least two similar rectal ACF chemoprevention trials (28) and NCT00176618. We found that Poly E (780 mg ECGC daily) failed to produce a statistically significant reduction in the primary endpoint of percent change in rectal ACF number comparing baseline to 6 months. Furthermore, aggregate ACF size did not differ significantly by study treatment arm. Thirty-one (79%) patients received at least 70% of the total dose over all months of treatment. At the end-of-treatment assessment, we also examined adenoma recurrence rates as a secondary endpoint. At 6 months, adenoma recurrence rates did not differ significantly by study arm. All end-of-treatment exams were limited to the left colon and rectum given the use of flexible sigmoidoscopy. In a randomized, non–placebo-controlled study in a Korean population, supplementation with a green tea extract (GTE) containing 206 mg of EGCG given daily for 12 months reduced the rate of metachronous colorectal adenomas from 42.3% to 23.6%, respectively, among 143 evaluable patients (9). Of note, no differences in body mass index, C-reactive protein levels or dietary intake were found by study arm. Another randomized, non–placebo-controlled trial from Japan found that GTE containing 157.5 mg of EGCG given daily for 12 months reduced the incidence of metachronous adenomas from 31% to 15% (3). Important differences are that our study evaluated ACF regression as the primary endpoint, and exposure to EGCG was limited to 6 months compared with 12 months in the studies from Korea and Japan. Furthermore, our study population consisted of higher risk patients who had prior advanced adenomas and not simply a prior colorectal adenoma. As in our study, negative results were obtained for Poly E supplementation in patients with low-grade cervical intraepithelial neoplasia whereby 98 women were randomized to receive either Poly E (containing 800 mg EGCG) or placebo once daily for 4 months (29). Chemoprevention studies have evaluated dosages of Poly E ranging from 200 mg to 800 mg daily. In a prior phase Ib study, Poly E at 400 and 600 mg, but not 200 mg, daily versus placebo for 6 months resulted in detectable concentrations (>10 pmol/g) of EGCG in the target organ, that is, esophageal mucosa (30). On the basis of our dose of EGCG at 780 mg daily, we would expect detectable EGCG concentrations in the target organ, that is, colonic mucosa, and the potential exists to measure ECGG concentrations using

Table 3. Toxicity monitoring ($n = 39$).

Adverse Event		Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)
Type	**Arm**				
Nausea	Poly-E	2 (10.5)			
	Placebo	1 (5.0)			
Abdominal pain	Placebo			1 (5.0[c])	
ALT[a] increase	Poly-E		1[c] (5.3)		
AST[b] increase	Poly-E		1[c] (5.3)		
Diarrhea	Poly-E	1 (5.3)			
Dizziness	Placebo		1 (5.0)		
Dyspepsia	Placebo			1 (5.0[c])	
Fatigue	Placebo		1 (5.0)		
Flu-like syndrome	Placebo		1 (5.0)		
Headache	Placebo	1 (5.0)			
Myalgia	Placebo		1 (5.0)		

[a]Alanine aminotransferase.
[b]Aspartate aminotransferase.
[c]Same patient.

study biopsy material from our clinical trial participants. Because it is stated that 8 ounces of brewed green tea typically contains about 50–100 mg of EGCG (3), the dose of 780 mg EGCG given in our study corresponds to approximately 10 cups per day, which has been associated with a reduced risk of colorectal cancer (4).

We collected colonoscopy and related pathology data on patients following study participation with a median follow-up time of 39.1 and 36.4 months in the treatment and placebo arms, respectively. Among 17 patients with available data who underwent surveillance colonoscopies post study treatment, recurrence of adenomas was found in 14.3% of patients previously treated with Poly E versus 40% for the placebo arm that was of borderline statistical significance. While these data suggest the possibility of a delayed suppressive effect of Poly E on adenoma recurrence, the small patient numbers warrant caution in their interpretation.

While natural dietary supplements like green tea are more acceptable to the general public than are pharmacologic agents for disease prevention, recipients of a standardized green tea extract, that is, Poly E, given in concentrated form must be carefully monitored for adverse effects. The main secondary objective of our study was to examine the safety and tolerability of Poly E in our study population. Poly E at the prescribed dose (1,200 mg daily) was well tolerated and no statistically significant increase in adverse events occurred in the treatment arm relative to the placebo arm. Adverse effects were generally mild and most were grade 1 or 2 events. One patient developed grade 2 hepatic transaminitis that resolved with cessation of Poly E. In a phase Ib trial of Poly E in patients with breast cancer, a dose of 800 mg daily was associated with a hepatic aminotransferase abnormality in 1 of 3 patients treated at that dose level (16). In another phase I trial of Poly E, patients with CLL received doses that were escalated from 400 to 2,000 mg twice daily and one-third of patients developed grade 1 transaminitis (11). These data suggest the relative safety of dose escalation of Poly E in future clinical studies.

The mechanisms underlying the antitumor efforts of EGCG include inhibition of EGFR and IGF/IGF1R signaling pathways as shown in animal and human studies (31, 32). Furthermore, studies in $Apc^{min/+}$ mice has shown that EGCG can inhibit WNT signaling whose function is nuclear translocation of β catechin to transcriptionally activate proto-oncogenes such as *c-myc*, *cyclin D1*, and *COX2* (33). Strengths of our study include the randomized, placebo-controlled and double-blinded design. Strict eligibility criteria were utilized for patient eligibility and endoscopic procedures were performed by nine expert endoscopists at two institutions. Study data monitoring included frequent toxicity assessments and safety evaluations. Endoscopic methodology was standardized and procedures were performed by endoscopists experienced in chromoendoscopy. Limitations include the relatively short duration of drug treatment and the limited sample size due to pending expiration of the study drug. We cannot exclude the possibility that a longer treatment duration could have shown a more pronounced inhibitory effect on rectal ACF. In conclusion, treatment with Poly E (1,200 mg daily/780 mg ECCG) for 6 months did not significantly reduce rectal ACF number in patients with prior advanced adenomas or colon cancer. Poly E was well tolerated and without significant toxicity at the dosage studied.

Authors' Disclosures

F.A. Sinicrope reports grants from NCI during the conduct of the study. J.B. Kisiel reports grants from Exact Sciences outside the submitted work; in addition, J.B. Kisiel has a patent for Detecting Colorectal Neoplasia 10370726 licensed and with royalties paid from Exact Sciences. P.J. Limburg reports other from Exact Sciences outside the submitted work; in addition, P.J. Limburg serves as Chief Medical Officer for Screening at Exact Sciences through a contracted services agreement with Mayo Clinic. P.J. Limburg and Mayo Clinic have contractual rights to receive royalties through this agreement. J.P. Meyers reports grants from NCI during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

F.A. Sinicrope: Conceptualization, funding acquisition, writing–original draft, writing–review and editing. **T.R. Viggiano:** Visualization, methodology. **N.S. Buttar:** Visualization, methodology. **L.M. Wong Kee Song:** Visualization, methodology. **K.W. Schroeder:** Visualization, methodology. **R.E. Kraichely:** Conceptualization, visualization, methodology. **M.V. Larson:** Conceptualization, visualization, methodology. **R.E. Sedlack:** Formal analysis, visualization, methodology. **J.B. Kisiel:** Formal analysis, visualization, methodology. **C.J. Gostout:** Visualization, methodology. **A.M. Kalaiger:** Writing–review and editing. **Á.V. Patai:** Visualization, project administration. **G. Della'Zanna:** Visualization, methodology, project administration. **A. Umar:** Visualization, methodology, project administration. **P.J. Limburg:** Conceptualization, visualization, methodology. **J.P. Meyers:** Formal analysis, visualization, methodology. **N.R. Foster:** Formal analysis, project administration. **C.S. Yang:** Conceptualization, visualization, methodology. **S. Sontag:** Visualization, methodology.

Acknowledgments

The study was supported by a grant (to F.A. Sinicrope) from the NCI (R01 CA132991).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 20, 2020; revised January 12, 2021; accepted February 23, 2021; published first March 1, 2021.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020;70:145–64.
2. Yang CS, Wang H. Cancer preventive activities of tea catechins. Molecules 2016;21:1679.
3. Shimizu M, Fukutomi Y, Ninomiya M, Nagura K, Kato T, Araki H, et al. Green tea extracts for the prevention of metachronous colorectal adenomas: a pilot study. Cancer Epidemiol Biomarkers Prev 2008;17: 3020–5.
4. Sun CL, Yuan JM, Koh WP, Yu MC. Green tea, black tea and colorectal cancer risk: a meta-analysis of epidemiologic studies. Carcinogenesis 2006;27:1301–9.
5. Ju J, Hong J, Zhou J-N, Pan Z, Bose M, Liao J, et al. Inhibition of intestinal tumorigenesis in Apcmin/+ mice by (-)-epigallocatechin-3-gallate, the major catechin in green tea. Cancer Res 2005;65:10623–31.
6. Xiao H, Hao X, Simi B, Ju J, Jiang H, Reddy BS, et al. Green tea polyphenols inhibit colorectal aberrant crypt foci (ACF) formation and prevent oncogenic changes in dysplastic ACF in azoxymethane-treated F344 rats. Carcinogenesis 2008;29:113–9.
7. Hao X, Xiao H, Ju J, Lee M-J, Lambert JD, Yang CS. Green tea polyphenols inhibit colorectal tumorigenesis in azoxymethane-treated F344 rats. Nutr Cancer 2017;69:623–31.
8. Adler DG, Gostout CJ, Sorbi D, Burgart LJ, Wang L, Harmsen WS. Endoscopic identification and quantification of aberrant crypt foci in the human colon. Gastrointest Endosc 2002;56:657–62.
9. Shin CM, Lee DHo, Seo AY, Lee HJ, Kim SB, Son W-C, et al. Green tea extracts for the prevention of metachronous colorectal polyps among patients who underwent endoscopic removal of colorectal adenomas: A randomized clinical trial. Clin Nutr 2018;37:452–8.
10. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res 2006;66:1234–40.
11. Shanafelt TD, Call TG, Zent CS, Leis JF, LaPlant B, Bowen DA, et al. Phase 2 trial of daily, oral Polyphenon E in patients with asymptomatic, Rai stage 0 to II chronic lymphocytic leukemia. Cancer 2013;119: 363–70.
12. Ju J, Lu G, Lambert JD, Yang CS. Inhibition of carcinogenesis by tea constituents. Semin Cancer Biol 2007;17:395–402.
13. Kumar N, Shibata D, Helm J, Coppola D, Malafa M. Green tea polyphenols in the prevention of colon cancer. Front Biosci 2007; 12:2309–15.
14. Chow H-HS, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, et al. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 2005;11:4627–33.
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. Crew KD, Brown P, Greenlee H, Bevers TB, Arun B, Hudis C, et al. Phase IB randomized, double-blinded, placebo-controlled, dose escalation study of polyphenon E in women with hormone receptor-negative breast cancer. Cancer Prev Res 2012;5:1144–54.
17. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med 2012; 366:687–96.
18. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 1987;37:147–51.
19. Roncucci L, Medline A, Bruce WR. Classification of aberrant crypt foci and microadenomas in human colon. Cancer Epidemiol Biomarkers Prev 1991;1:57–60.
20. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG, Jurcisek JA, et al. Aberrant crypts: putative preneoplastic foci in human colonic mucosa. Cancer Res 1991;51:1564–7.
21. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, et al. Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 1998;339:1277–84.
22. Shpitz B, Bomstein Y, Mekori Y, Cohen R, Kaufman Z, Neufeld D, et al. Aberrant crypt foci in human colons: distribution and histomorphologic characteristics. Hum Pathol 1998;29:469–75.
23. Bouzourene H, Chaubert P, Seelentag W, Bosman FT, Saraga E. Aberrant crypt foci in patients with neoplastic and nonneoplastic colonic disease. Hum Pathol 1999;30:66–71.
24. Otori K, Sugiyama K, Hasebe T, Fukushima S, Esumi H. Emergence of adenomatous aberrant crypt foci (ACF) from hyperplastic ACF with concomitant increase in cell proliferation. Cancer Res 1995;55: 4743–6.
25. Yokota T, Sugano K, Kondo H, Saito D, Sugihara K, Fukayama N, et al. Detection of aberrant crypt foci by magnifying colonoscopy. Gastrointest Endosc 1997;46:61–65.
26. Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, et al. Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program. J Cell Biochem Suppl 1994;20:32–54.
27. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975; 31:103–15.
28. Limburg PJ, Mahoney MR, Ziegler KLA, Sontag SJ, Schoen RE, Benya R, et al. Randomized phase II trial of sulindac, atorvastatin, and prebiotic dietary fiber for colorectal cancer chemoprevention. Cancer Prev Res 2011;4:259–69.
29. Garcia FAR, Cornelison T, Nuño T, Greenspan DL, Byron JW, Hsu C-H, et al. Results of a phase II randomized, double-blind, placebo-controlled trial of Polyphenon E in women with persistent high-risk HPV infection and low-grade cervical intraepithelial neoplasia. Gynecol Oncol 2014;132:377–82.
30. Joe AK, Schnoll-Sussman F, Bresalier RS, Abrams JA, Hibshoosh H, Cheung K, et al. Phase Ib randomized, double-blinded, placebo-controlled, dose escalation study of polyphenon E in patients with Barrett's Esophagus. Cancer Prev Res 2015;8:1131–7.
31. Adachi S, Nagao T, Ingolfsson HI, Maxfield FR, Andersen OS, Kopelovich L, et al. The inhibitory effect of (-)-epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. Cancer Res 2007;67: 6493–501.
32. Shimizu M, Shirakami Y, Sakai H, Tatebe H, Nakagawa T, Hara Y, et al. EGCG inhibits activation of the insulin-like growth factor (IGF)/IGF-1 receptor axis in human hepatocellular carcinoma cells. Cancer Lett 2008;262:10–18.
33. Zhang Y, Hays A, Noblett A, Thapa M, Hua DH, Hagenbuch B. Transport by OATP1B1 and OATP1B3 enhances the cytotoxicity of epigallocatechin 3-O-gallate and several quercetin derivatives. J Nat Prod 2013;76:368–73.