VOLUME 27 · NUMBER 23 · AUGUST 10 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase I Trial of Daily Oral Polyphenon E in Patients With Asymptomatic Rai Stage 0 to II Chronic Lymphocytic Leukemia

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Submitted November 26, 2008; accepted February 9, 2009; published online ahead of print at www.jco.org on May 26, 2009.

Supported by grants from the National Cancer Institute (Grants No. CA113408 and CA6912), the CLL Global Research Foundation, CLL Topics, Commonwealth Foundation for Cancer Research, Polyphenon E International, and the Mayo Clinic Cancer Center.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/09/2723-3808/$20.00

DOI: 10.1200/JCO.2008.21.1284

ABSTRACT

Purpose
To define the optimal dose of Polyphenon E for chronic daily administration and tolerability in patients with chronic lymphocytic leukemia (CLL).

Patients and Methods
Previously untreated patients with asymptomatic Rai stage 0 to II CLL were eligible for participation. Polyphenon E with a standardized dose of epigallocatechin-3-gallate (EGCG) was administered using the standard phase I design with three to six patients per dose level (range, 400 to 2,000 mg by mouth twice a day). Trough plasma EGCG levels were measured 1 month after initiation of therapy. Response was classified using the National Cancer Institute (NCI) Working Group (WG) Criteria.

Results
Thirty-three eligible patients were accrued to dose levels 1 to 8. The maximum-tolerated dose was not reached. The most common adverse effects included transaminitis (33%, all grade 1), abdominal pain (30% grade 1, 0% grade 2, and 3% grade 3), and nausea (39% grade 1 and 9% grade 2). One patient experienced an NCI WG partial remission. Other signs of clinical activity were also observed, with 11 patients (33%) having a sustained ≥ 20% reduction in absolute lymphocyte count (ALC) and 11 (92%) of 12 patients with palpable adenopathy experiencing at least a 50% reduction in the sum of the products of all nodal areas during treatment. Trough plasma EGCG levels after 1 month of treatment ranged from 2.9 to 3,974 ng/mL (median, 40.4 ng/mL).

Conclusion
Daily oral EGCG in the Polyphenon E preparation was well tolerated by CLL patients in this phase I trial. Declines in ALC and/or lymphadenopathy were observed in the majority of patients. A phase II trial to evaluate efficacy using 2,000 mg twice a day began in November 2007.

J Clin Oncol 27:3808-3814. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common subtype of leukemia in the United States and is presently an incurable disease. Most patients with CLL are now identified with early-stage disease when they are found to have an elevated lymphocyte count on a blood count obtained for other reasons.[1] Unfortunately, 50% of patients with early-stage disease have a biologically aggressive form of CLL that progresses[2-5] and leads to premature death.[2] Unlike most other malignant disorders in which diagnosis at an early stage increases the probability that a patient can be cured, treatment of unselected early-stage CLL patients with cytotoxic chemotherapy is associated with increased toxicity but no increase in survival.[6,7] Thus, there is no effective intervention to alter the course of disease for patients with early-stage CLL at the present time.[8]

Green tea has long been touted as a health-promoting substance. Recent scientific investigations have identified the active chemical compounds in green tea designated tea polyphenons or catechins.[9,10] Epigallocatechin-3-gallate (EGCG) is the major catechin in tea. A number of epidemiologic studies have linked consumption of green tea to decreased risk of cancer, and animal models have supported green tea's ability to prevent tumorigenesis.[9,10] A number of mechanism have been

proposed for this effect of green tea including antiangiogenic properties,[11,12] cell cycle arrest,[9,10,13] effects on folate metabolism,[14] effects on DNA damage,[15] inhibition of telomerase,[16,17] reductions in level of antiapoptotic proteins,[18] proteasome inhibition,[19,20] generation of reactive oxygen species,[14,21-24] and demethylating/epigenetic effects.[25-27] Minimal toxicity ($\leq$ grade 1) has been observed in clinical trials administering EGCG to healthy adults at doses of 800 mg/d.[28] In other trials of green tea extracts in patients with advanced malignancy, doses as high as 1 g/m^2 three times per day were tolerated for up to 6 months.[29]

We recently investigated the effect of EGCG on CLL B cells in vitro.[30] EGCG induced caspase-dependent death in eight of 10 CLL samples.[30] Treatment of CLL B cells with EGCG at doses as low as 325 ng/mL induced significant downregulation of antiapoptotic proteins (MCL1 and XIAP) known to increase the resistance of CLL B cells to apoptosis.[30] EGCG also decreased vascular endothelial growth factor (VEGF) receptor (R1 and R2) phosphorylation and blocked VEGF-induced increases in MCL1 and XIAP.

After the publication of these findings, some patients with CLL and other low-grade lymphomas began using over-the-counter products containing tea polyphenols of their own initiative. We observed that several of these patients with low-grade B-cell malignancies had an objective clinical response.[31] These observations, together with the preclinical findings[30] and the known favorable toxicity profile of EGCG in human patients,[28] provided a strong rational for a clinical trial of EGCG in patients with asymptomatic Rai stage 0 to II CLL.

PATIENTS AND METHODS

Patient Eligibility

Previously untreated patients with asymptomatic Rai stage 0 to II CLL who did not meet National Cancer Institute Working Group (NCI WG)[8] criteria to initiate chemotherapy were eligible for participation. The study was designed as a phase I/II trial with results of the phase I study reported here. Patients were required to have a confirmed diagnosis of CLL by standard criteria.[8] Mantle cell lymphoma was excluded in all patients by fluorescent in situ hybridization (FISH) assessing for a t(11;14).

The eligibility criteria required participants to have an Eastern Cooperative Oncology Group performance status of 0 to 2, a life expectancy of $\geq$ 6 months, adequate renal function (creatinine $\leq$ 1.5$\times$ the upper limit of normal or creatinine clearance $\geq$ 40 mL/min), and a total bilirubin less than 1.5$\times$ the upper limit of normal (unless a result of Gilbert's disease). Concurrent chemotherapy, immunotherapy, radiotherapy, or corticosteroid treatment was not allowed. Patients were also not eligible if they had used over-the-counter green tea or green tea extracts with medicinal intent within 8 weeks of registration (half-life of EGCG is approximately 4 hours[28]). The protocol was reviewed and approved by the Mayo Clinic Institutional Review Board and registered with the National Institutes of Health (clinicaltrials.gov). All patients provided written informed consent before study enrollment in accordance with the Declaration of Helsinki.

Protocol Treatment

Polyphenon E capsules (Polyphenon Pharma, New York, NY) containing approximately 200 mg of EGCG were supplied by the NCI. The phase I portion of the trial was designed to have up to eight dose levels using the standard phase I design of three to six patients per dose level. In December 2005, the NCI requested that dose escalation be temporarily suspended at dose level 2 to allow the US Food and Drug Administration (FDA) to review the results of animal studies evaluating EGCG toxicity. In the interim, additional patients were accrued at dose level 2 to better define the tolerability of 800 mg twice per day.

The trial was originally designed to administer Polyphenon E in the fasting state. In February 2006, the FDA temporarily suspended all human trials of EGCG to allow additional review of toxicity data. This led to interruption of treatment for regulatory reasons for eight patients on dose levels 1 and 2 after 2 to 5 months of treatment. The FDA later allowed trials of Polyphenon E to resume but mandated that all US clinical trials administer drug with food. Accordingly, the protocol was modified, and Polyphenon E was administered with food for dose levels 3 to 8. Response in the eight patients affected by this interruption was assessed on the date of interruption, at which point these individuals were censored for response analysis (six patients went off study, and two later resumed treatment after approximately a 5-month interruption).

All study participants were provided a medication diary to indicate the time and quantity of medication usage, which was reviewed at each follow-up visit. Patients remained on active treatment (dose range, 400 to 2,000 mg by mouth twice daily) for up to 6 months and were evaluated once every 4 weeks by physical examination and laboratory testing. Treatment was discontinued in the event of progressive disease, as defined by the NCI WG criteria,[8] or excessive toxicity. At the completion of 6 months of active treatment, patients went to observation. With the approval of the treating hematologist, patients who had not experienced disease progression and who desired to remain on EGCG were provided EGCG-containing capsules at their assigned dose level for up to 12 additional months.

Toxicity was graded using NCI Common Terminology Criteria for Adverse Events version 3.0. Because there is a low threshold for toxicity in the treatment of patients not meeting standard criteria for progressive disease, cycle 1 adverse events more than grade 1 attributed to Polyphenon E that did not respond to supportive care were considered dose-limiting toxicity (DLT). Transient toxicity more than grade 1 that responded to supportive care was recorded as an adverse event but was not considered dose limiting. In general, for grade 2 adverse events attributed to Polyphenon E, therapy was held until symptoms resolved to $\leq$ grade 1 and then reinitiated along with supportive care measures at the same dose level. Because of the mandate of the FDA, the notable exception to this approach was the response to any elevation in transaminases (AST or ALT), in which case Polyphenon E was held for grade 1 adverse events regardless of attribution until these values returned to normal. Once the patient had normal levels, Polyphenon E was then reinitiated at the same dose level. If grade 1 transaminitis recurred, Polyphenon E was held and reinitiated at the next lower dose once AST or ALT values had returned to normal. Regardless of attribution, patients with $\geq$ grade 2 transaminitis were required by the FDA to permanently discontinue study treatment. For grade 3 to 4 adverse events attributed to study treatment or recurrent grade 2 events, Polyphenon E was held until symptoms resolved to $\leq$ grade 1 and then reinitiated at the next lower dose level along with supportive care measures. The maximum-tolerated dose (MTD) was defined as the dose level below the lowest dose that induced a DLT in at least one third of patients (at least two of six patients) after one cycle of therapy.

Risk Stratification Parameters

All patients underwent a comprehensive CLL prognostic evaluation including assessment of CD38 and ZAP-70 expression, FISH detectable cytogenetic defects, and *IgVH* gene mutation testing as previously described.[32-34]

Criteria for Response

Best response during the 6 months of active therapy was evaluated using the NCI WG criteria.[8] Given the favorable toxicity profile of Polyphenon E in healthy adults[12] and the intention to evaluate the efficacy of this agent to delay or prevent disease progression in CLL, we also evaluated an additional response category termed biologic response. This category was prospectively defined in the study protocol after discussion and approval of this end point by the NCI because of recognition that anticancer botanicals such as Polyphenon E may work through unique, noncytotoxic mechanisms. These effects could be clinically beneficial by stabilizing disease rather than causing tumor regression. To be classified a biologic response, a reduction in the absolute lymphocyte count (ALC) of more than 20% from the pretreatment level for at least 2 months or a $\geq$ 30% reduction in all palpable lymphadenopathy without meeting the NCI WG criteria for partial remission (PR) was required.

Plasma Polyphenol Levels

Trough (approximately 12 hours after last dose) total plasma EGCG levels were measured at the end of the first month of therapy. Blood was collected into Vacutainer tubes (BD, Franklin Lakes, NJ) containing sodium heparin, and plasma was removed after centrifugation. Plasma was then aliquoted into cryotubes containing a small aliquot of ascorbate-EDTA solution as described by Lee et al[35] and stored at −80°C until analysis. Plasma levels of EGCG were then measured in the laboratory of the authors (C.S.Y.) using an established high-performance liquid chromatography procedure with a Coulochem (ESA, Chelmsford, MA) electrode array system.[36]

Statistical Analysis

The primary outcome for this phase I trial was the number of DLTs and determination of the MTD, with clinical response the secondary outcome. Confirmed clinical responses (NCI WG complete remission or PR) on two consecutive evaluations at least 4 weeks apart and biologic responses, as defined earlier, were used as measures of clinical response and were summarized by simple descriptive statistics. Differences in response by key patient characteristics (ie, ZAP-70 status) were compared using the Fisher's exact test. Correlations between plasma EGCG level and dose/response were evaluated with the Spearman rank coefficient and the Wilcoxon rank sum test where appropriate.

RESULTS

Patient Demographics

Thirty-six patients were enrolled onto the study between August 2005 and September 2007. Median time from diagnosis to registration was 30.5 months (range, 0 to 170 months). Three patients were considered ineligible for evaluation because they took Polyphenon E incorrectly during cycle 1 (eg, took half the prescribed dose), which precluded accurate assessment of toxicity at the prescribed dose. The clinical characteristics of the 33 eligible patients are listed in Table 1. Most patients had favorable prognostic profiles on FISH, ZAP-70, CD38, and *IgVH* gene mutation analysis at study entry consistent with the eligibility requirements that patients be asymptomatic and have earlier stage disease.

Table 1. Patient Demographics and Clinical Characteristics

Patient Demographics and Clinical Characteristics	No. of Patients (N = 33)	%
Age, years		
Median	62	
Range	41-76	
Male	24	73
ALC, $\times 10^9$/L		
Median	35.1	
Range	5.4-355	
Rai stage		
0	18	55
I	11	33
II	4	12
ZAP-70 ≥ 20%	8	24
CD38* ≥ 30%	4	13
IgVH† unmutated	8	24
FISH		
del(13q14.2)	20	61
Normal	9	27
Trisomy 12	4	12

Abbreviations: ALC, absolute lymphocyte count; FISH, fluorescent in situ hybridization.
*Available for 32 of 33 patients.
†Although *IgVH* mutation testing was performed in all patients, two patients could not be sequenced and hence had uninterpretable *IgVH* test results.

Toxicity and Tolerability

Median overall compliance with the prescribed dose of Polyphenon E as assessed using pill diaries was 99% (range, 55% to 104%). The MTD was not reached after completing accrual to dose levels 1 to 8. Only two patients experienced a DLT. A grade 2 dysphagia considered to be a DLT was observed during cycle 1 in one of six patients enrolled at dose level 4. In the second case, a patient on dose level 8 experienced grade 2 sweating, flatulence, abdominal distention, and nausea during cycle 1, which were all considered dose limiting. The number of DLTs by dose level is shown in Table 2.

Non–dose-limiting adverse effects during the 6 months of active treatment were generally mild. When examining all adverse events at least possibly attributed to therapy, five patients (15%) reported a maximum of a grade 2 event, and two patients (6%) reported a grade 3 event. The frequencies of specific adverse effects at least possibly attributed to therapy by Common Terminology Criteria for Adverse Events classification are listed in Table 3. Twenty-one patients (64%) completed six cycles of active therapy. Twelve patients discontinued therapy early; two refused further treatment, three experienced an adverse event, two experienced progression, one reported a new primary malignancy (cholangiocarcinoma), and four elected not to resume treatment when the temporary FDA suspension of all EGCG trials ended (see Patients and Methods).

Response to Therapy

One NCI WG PR was observed at dose level 8. In addition, a majority of patients had a reduction in ALC (Table 4). Reductions in ALC were transient in some patients, whereas in others, a steady, sustained stepwise reduction was observed throughout the 6 months of active therapy. Among the 15 patients (45%) who had a ≥ 20% reduction in ALC, 11 (73%) had a sustained decrease ≥ 20% for at least 2 months that fulfilled the criteria for biologic response. In a

Table 2. DLT

Dose Level	Dose (mg bid)	Total No. of Patients	No. of Patients With DLT
1	400	3	0
2	800	6*	0
3	1,000	3	0
4	1,200	6	1
5	1,400	3	0
6	1,600	3	0
7	1,800	3	0
8	2,000	6	1

NOTE. Cycle 1 adverse events > grade 1 attributed to Polyphenon E that did not respond to supportive care were considered DLTs. Transient adverse events that responded to supportive care were recorded as adverse events but were not considered dose limiting (see Table 3).
Abbreviations: DLT, dose-limiting toxicity; bid, twice per day.
*Although no DLTs were observed among the first three patients accrued to dose level 2, the National Cancer Institute (NCI) temporarily suspended dose escalation to allow the US Food and Drug Administration to review the results of animal studies evaluating epigallocatechin-3-gallate toxicity. During this suspension, three additional patients were accrued at dose level 2 to better define tolerability at this dose level before receiving approval from the NCI to resume dose escalation (see Patients and Methods).

Table 3. Adverse Effects at Least Possibly Related to Treatment

Adverse Effect*	Grade 1		Grade 2		Grade 3	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
Nausea	13	39	3	9	0	0
Abdominal pain	10	30	0	0	1	3
Transaminitis	11	33	0	0	0	0
Anorexia	9	27	2	6	0	0
Diarrhea	6	18	2	6	1	3
Dyspepsia	12	36	0	0	0	0
Flatulence	7	21	2	6	0	0
Fatigue	8	24	1	3	0	0
Hyperglycemia	1	3	0	0	0	0

*Classified according to the National Cancer Institute Common Terminology Criteria of Adverse Events.

per-protocol analysis of patients who completed six cycles of therapy, the ALC at the completion of therapy was less than baseline in 11 (52%) of 21patients. Among the 12 patients with palpable adenopathy at enrollment, 11 (92%) experienced at least a 50% reduction in the sum of the products of all palpable nodal areas at some point during treatment. Overall, 18 (55%) of 33 patients fulfilled the criteria for a biologic response based on attaining either a sustained $\geq$ 20% decline in ALC or a $\geq$ 30% reduction in the sum of the products of all nodal areas at some point during the 6 months of active treatment. Achieving a biologic response appeared to be related to dose level; two (17%) of 12 patients treated on dose levels 1 to 3 achieved a biologic response compared with 16 (76%) of 21 patients treated on dose levels 4 to 8 (Table 5).

Correlative Prognostic Factors and Treatment Outcomes

The proportions of patients with a PR or biologic response by each prognostic parameter are listed in Table 6. Although no differences in response were observed based on *IgVH*, ZAP-70, or CD38, individuals with trisomy 12 on FISH appeared less likely to achieve a response ($P = .04$).

Plasma Polyphenon Levels

Trough total plasma EGCG levels after 1 month of therapy ranged from 2.9 to 3,974 ng/mL (median, 40.4 ng/mL). Plasma levels did not clearly correlate with dose, the likelihood of attaining a PR/biologic response, or the degree of reduction in ALC after 1 month of treatment (Fig 1), suggesting that sensitivity to Polyphenon E may relate more to characteristics of the leukemic clone rather than the EGCG plasma level achieved.

DISCUSSION

Daily oral EGCG in the Polyphenon E preparation was well tolerated up to the maximum dose tested (2,000 mg orally twice per day) in this phase I trial for patients with asymptomatic Rai stage 0 to II CLL. The most severe toxicity was $\leq$ grade 1 for more than 80% of patients, and only two (6%) of 33 patients experienced $\geq$ grade 3 toxicity. Importantly, signs of clinical activity were observed, with one patient achieving an NCI WG PR and more than 50% of study patients attaining a sustained decline in ALC of $\geq$ 20% and/or a $\geq$ 50% reduction in lymphadenopathy at some point during treatment. Clinical activity seemed to be related to dose because more than 75% of patients treated on dose levels 4 to 8 (1,200 to 2,000 mg twice per day) achieved at least a biologic response compared with less than 20% of patients treated on dose levels 1 to 3.

Despite extensive data demonstrating that 50% to 70% of patients with asymptomatic, earlier stage CLL will experience progression and require treatment,[2,6,37] such patients are presently managed with observation.[38] The phase I data presented here suggests that Polyphenon E may have clinical activity in these patients, and the fact that this treatment is very well tolerated suggests that Polyphenon E merits additional evaluation as a disease-stabilizing agent. With the

Table 4. Best Response in ALC and Nodes

Response	No. of Patients	%
Best reduction in ALC		
At least 10% decline	26	79
At least 20% decline	15	45
At least 30% decline	10	30
At least 40% decline	5	15
At least 50% decline	2	6
Best reduction in nodes		
At least 50% reduction in sum of products	11*	92

Abbreviation: ALC, absolute lymphocyte count.
*Twelve patients had palpable lymphadenopathy at study entry.

Table 5. Response by Dose Level

Dose Level	Dose (mg bid)	No. of Patients	No. of Patients With Partial or Biologic Response
1	400	3	1
2	800	6	0
3	1,000	3	1
4	1,200	6	4
5	1,400	3	3
6	1,600	3	2
7	1,800	3	2
8	2,000	6	5

Abbreviation: bid, twice per day.

Table 6. Biologic Response by Prognostic Parameters

Prognostic Parameter	No. of Patients	Patients With at Least Biologic Response		*P*
		No.	%	
ZAP-70				.70
Negative (< 20%)	25	13	52	
Positive (≥ 20%)	8	5	63	
CD38				1.0
Negative (< 30%)	28	15	54	
Positive (≥ 20%)	4	2	50	
IgVH				1.0
Mutated	23	13	57	
Unmutated	8	4	50	
FISH				.04
del(13q14.2)	20	11	55	
Normal	9	7	78	
Trisomy 12	4	0	0	

Abbreviation: FISH, fluorescent in situ hybridization.

exception of trisomy 12, the clinical activity observed with Polyphenon E in the present trial did not seem to be influenced by ZAP-70, CD38, or *IgVH* gene mutation status. This observation suggests that Polyphenon E may have activity in Rai stage 0 to II patients whose prognostic parameters suggest they are at high risk for disease progression.[39]

The mechanism(s) by which EGCG affects CLL B cells in vivo is unknown. In vitro studies of CLL B cells show that EGCG decreases VEGF receptor phosphorylation and downregulates the expression of MCL-1 and XIAP.[30] Indeed, EGCG seems to have greater ability to antagonize MCL-1 than other BCL-2 family member inhibitory compounds in development as CLL therapies including gossypol, apogossypol, and ABT 737.[40] Other studies suggest that EGCG is a potent inhibitor of BCL-2 at nanomolar concentrations.[18] Although these mechanisms are of particular interest because of the importance of BCL-2 family members,[41-43] and particularly MCL-1,[44-47] in CLL B-cell apoptotic resistance, numerous other mechanisms of action have been proposed for the antitumor effects of EGCG observed in vitro.[9-27,40,48]

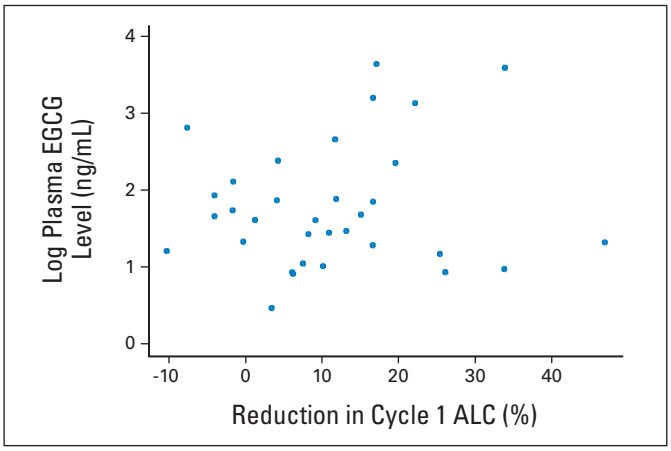

Fig 1. Plasma levels of epigallocatechin-3-gallate (EGCG) and response. Figure shows the relationship between absolute lymphocyte count (ALC) reduction at 1 month (*x*-axis) and trough plasma EGCG level (*y*-axis) at 1 month. EGCG plasma levels are shown on a log normal scale as a result of the wide variation in plasma levels.

Because in vitro studies suggest EGCG may have activity against a wide range of human malignancies including prostate, colon, lung, and breast cancer,[49-52] the results of this phase I trial may have relevance for clinical studies of EGCG in other diseases. In this respect, it should be noted that the median age of patients in our study was more than 60 years old and that 70% of participants were men. Although the MTD was not reached in our trial, we chose not to escalate beyond the 2,000 mg twice per day dose level because the high number of capsules required to achieve this dose (10 capsules twice per day) made further dose escalation problematic. It may be possible to escalate beyond this dose level if a more concentrated preparation becomes available. Notably, the plasma levels attained on dose levels 3 to 8 in our study were achieved administering Polyphenon E in the fed state, whereas fasting administration would likely allow patients to achieve similar or higher plasma levels at lower Polyphenon E doses.[53]

The reason for the wide variation in trough plasma EGCG levels observed in our study is unknown. This variation could relate to differences in absorption when administered with food (eg, total calories, proportion of fat/protein/carbohydrate), metabolism, or protein binding. It is also notable that trough plasma EGCG levels did not clearly relate to the clinical activity of Polyphenon E observed among CLL patients. This observation could indicate other pharmacokinetic parameters (eg, peak levels, area under the curve, and so on) or characteristics of the leukemic clone may relate to the clinical effects of Polyphenon E in CLL patients more strongly than trough plasma levels.

In conclusion, daily oral EGCG in the Polyphenon E preparation was well tolerated at doses up to 2,000 mg twice per day for up to 6 months in patients with asymptomatic Rai stage 0 to II CLL. Declines in ALC and lymphadenopathy during Polyphenon E therapy were observed in the majority of patients. A phase II trial evaluating the efficacy of Polyphenon E (2,000 mg twice per day) in patients with asymptomatic Rai stage 0 to II CLL was initiated in November 2007.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Neil E. Kay, Polyphenon E International **Stock Ownership:** None **Honoraria:** Neil E. Kay, Polyphenon E International **Research Funding:** Tait D. Shanafelt, Polyphenon E International; Chung S. Yang, Polyphenon E International; Neil E. Kay, Polyphenon E International **Expert Testimony:** None **Other Remuneration:** None

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