Original Article

Phase 2 Trial of Daily, Oral Polyphenon E in Patients With Asymptomatic, Rai Stage 0 to II Chronic Lymphocytic Leukemia

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BACKGROUND: The objective of the current study was to follow up the results of phase 1 testing by evaluating the clinical efficacy of the green tea extract Polyphenon E for patients with early stage chronic lymphocytic leukemia (CLL). **METHODS:** Previously untreated patients with asymptomatic, Rai stage 0 to II CLL and an absolute lymphocyte count (ALC) $\geq 10 \times 10^9$/L were eligible for this phase 2 trial. Polyphenon E with a standardized dose of epigallocatechin gallate (EGCG) (2000 mg per dose) was administered twice daily. **RESULTS:** A total of 42 patients received Polyphenon E at a dose of 2000 mg twice daily for up to 6 months. Of these patients, 29 (69%) had Rai stage I to II disease. Patients received a median of 6 cycles of treatment (range, 1 cycle-6 cycles). The most common grade 3 side effects (according to National Cancer Institute Common Terminology Criteria for Adverse Events) were transaminitis (1 patient), abdominal pain (1 patient), and fatigue (1 patient). Clinical activity was observed, with 13 patients (31%) experiencing a sustained reduction of $\geq$ 20% in the ALC and 20 of 29 patients (69%) with palpable adenopathy experiencing at least a 50% reduction in the sum of the products of all lymph node areas. EGCG plasma levels after 1 month of therapy were found to be correlated with reductions in lymphadenopathy (correlation co-efficient, 0.44; $P = .02$). Overall, 29 patients (69%) fulfilled the criteria for a biologic response with either a sustained decline $\geq$ 20% in the ALC and/or a reduction $\geq$ 30% in the sum of the products of all lymph node areas at some point during the 6 months of active treatment. **CONCLUSIONS:** Daily oral EGCG in the Polyphenon E preparation was well tolerated by patients with CLL in this phase 2 trial. Durable declines in the ALC and/or lymphadenopathy were observed in the majority of patients. ***Cancer* 2012;000:000–000.** © *2012 American Cancer Society.*

KEYWORDS: chronic lymphocytic leukemia, treatment, prognosis, green tea, early stage.

INTRODUCTION

Green tea has long been proposed as a health-promoting substance that reduces the risk of cancer.[1,2] After 3 case-control studies demonstrated that green tea intake was associated with a reduced risk of leukemia[3,4] and non-Hodgkin lymphoma,[5] a population-based cohort study of approximately 42,000 individuals prospectively followed for 9 years was conducted.[6] Green tea consumption was inversely associated with the risk of lymphoid malignancies even after adjusting for 16 other personal characteristics including age, sex, smoking history, level of education, occupation, consumption of other dietary products, and family history of leukemia.[6]

Tea polyphenols (catechins) exert multitargeted effects on malignant cells.[7-10] Epigallocatechin gallate (EGCG), the major catechin in tea, induces apoptotic cell death in animal models of human non-Hodgkin lymphoma,[11] B-cell lymphoma cell lines,[12,13] and primary chronic lymphocytic leukemia (CLL) B cells.[14] Subsequent case reports in patients with low-grade B-cell malignancies suggested these preclinical findings may have clinical relevance.[15]

Based on this series of observations and the favorable toxicity profile of green tea extracts reported in human testing,[16,17] we conducted a phase 1/2 trial of daily, oral Polyphenon E (a standardized, pharmaceutical-grade catechin preparation) for patients with asymptomatic, Rai stage 0 to II CLL.[18] As previously reported, daily oral EGCG was well tolerated at the maximum dose tested (2000 mg twice per day) in the phase 1 component of the trial and declines in the absolute lymphocyte count (ALC) and/or lymphadenopathy were observed in the majority of the 36 patients treated.[18] Coincident with our CLL trial, a randomized, placebo control trial of green tea catechins in patients with high-grade prostate intraepithelial neoplasia found that tea catechins reduced the risk of progression to prostate cancer (progression to

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prostate cancer at 1 years and 2 years: tea catechins, 3% and 11%, respectively; placebo, 30% and 53%, respectively; $P < .01$).[19,20] Similar studies have also suggested EGCG may result in a clinical benefit in patients with high-risk premalignant oral lesions.[21] These studies extend the extensive preclinical evidence and suggest that green tea catechins may have clinical benefits in patients with premalignant and indolent malignant conditions. In the current study, we report the results of the phase 2 component of the CLL trial exploring the clinical benefits of Polyphenon E.

MATERIALS AND METHODS

Patient Eligibility

We instituted a phase 1/2 trial of Polyphenon E in patients with previously untreated asymptomatic, Rai stage 0 to II CLL who did not meet National Cancer Institute Working Group(NCI-WG)[22] criteria to initiate chemotherapy. Patients were required to have a confirmed diagnosis of CLL by standard criteria.[22] Mantle cell lymphoma was excluded in all patients by fluorescence in situ hybridization (FISH) assessing for a t(11;14). The eligibility criteria for the phase 2 portion of the trial were identical to those reported for the phase 1 portion of the study[18] with 2 exceptions. First, although patients who had used over-the-counter green tea or green tea extracts with medicinal intent were eligible for the phase 1 portion of the trial provided they had not used such medications within 8 weeks of registration; such individuals were excluded from the phase 2 portion of the trial so that it better reflected the experience of patients who had not been previously treated with green tea extracts. Second, the phase 1 portion of the trial required patients have an absolute lymphocyte count (ALC) of at least 5×10^9/L whereas the phase 2 portion of the trial required patients have an ALC of at least 10×10^9/L to allow for the more accurate characterization of changes in the ALC. The protocol was reviewed and approved by the Mayo Clinic Institutional Review Board and registered with the National Institute 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 containing approximately 200 mg of EGCG were supplied by the NCI or directly by Polyphenon E International, Inc (New York, NY). All patients in the phase 2 portion of the trial received Polyphenon E at a dose of 1000 mg orally twice per day for the first 7 days of cycle 1, at which point the dose was increased to 2000 mg orally twice per day. Polyphenon E was administered with a light meal/snack. All study subjects were provided with a medication diary to indicate the time and quantity of medication usage that was reviewed at each follow-up visit. Patients remained on active treatment 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 excessive toxicity or progressive disease (PD) as defined by the NCI-WG criteria.[8] At the completion of 6 months of active treatment, patients entered observation. With the approval of the treating hematologist, patients who had not experienced disease progression and who desired to remain on treatment were provided with Polyphenon E 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 tolerance for toxicity in the treatment of patients with CLL who do not meet standard criteria for PD, dose modifications were required for adverse events of $>$ grade 1 that were attributed to Polyphenon E and that did not respond to supportive care. In general, for patients with grade 2 adverse events attributed to Polyphenon E, therapy was withheld until symptoms resolved to $\leq$ grade 1, at which time therapy was reinitiated at the original dose along with supportive care measures. For patients with adverse events of grade 3 to 4 that were attributed to study treatment or recurrent grade 2 events, Polyphenon E was withheld until symptoms resolved to $\leq$ grade 1 and then was reinitiated at the next lower dose level (reduction of 200 mg per dose) along with supportive care measures. Due to a mandate from the US Food and Drug Administration (FDA), the notable exception to this approach was the response to any elevation in transaminases (aspartate aminotransferase [AST] or alanine aminotransferase [ALT]), in which case Polyphenon E was withheld for grade 1 adverse events regardless of attribution until these values returned to normal, at which time Polyphenon E was reinitiated at the same dose level. If grade 1 transaminitis recurred, Polyphenon E was withheld and reinitiated at the next lower dose (a reduction of 200 mg twice daily) 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.

Risk Stratification Parameters

All patients underwent a comprehensive CLL prognostic evaluation including assessment of cluster of differentiation 38 (CD38) and zeta-chain–associated protein kinase

70 (ZAP-70) expression, FISH-detectable cytogenetic defects, and immunoglobulin heavy chain variable region (IGHV) gene mutation testing as previously described.[23,24]

Criteria for Response

The best response during the 6 months of active therapy was evaluated using the NCI-WG criteria.[22] Given the favorable toxicity profile of Polyphenon E in healthy adults[16] and the intention to evaluate the efficacy of this agent to delay or prevent PD in patients with CLL, we also evaluated an additional response category termed "biologic response" among patients who did not meet standard NCI-WG criteria for complete or partial response. The criteria for a biologic response were prospectively defined in the study protocol after discussion and approval of this endpoint by the NCI because of recognition that anticancer botanicals such as Polyphenon E may work through unique, noncytotoxic mechanisms. These criteria were evaluated and published for the patients participating in the phase 1 trial[18] before the phase 2 trial was initiated. Biologic response was defined as a reduction in the ALC of $>$ 20% from the pretreatment level that was sustained for at least 2 months or a $\geq$ 30% reduction in all palpable lymphadenopathy. Biologic response evaluation was included as a primary endpoint in the study protocol with NCI approval to ensure that the study assessed the potential ability of EGCG to prevent CLL progression in patients who did not achieve a conventional response.

Plasma Polyphenol Levels

Trough (approximately 12 hours after the last dose) plasma EGCG levels were measured at the end of the first month of therapy by one of the authors (C.S.Y.) using an established high-performance liquid chromatography procedure with a Coulochem electrode array system (Thermo Scientific, Chelmsford, Mass).[25]

Statistical Analysis

The primary outcomes for this phase 2 trial were the assessment of tolerability and clinical response. Confirmed clinical responses (NCI-WG complete or partial remission) on 2 consecutive evaluations (eg, over a 2-month interval) and biologic responses as defined earlier were used as measures of clinical response and were summarized using simple descriptive statistics. Differences in response by key patient characteristics (ie, ZAP-70 status) were compared using the Fisher exact test. Correlations between the plasma EGCG level and dose/response were evaluated with the Spearman rank coefficient and the Wilcoxon rank sum test when appropriate. Treatment-free survival was defined as the time from the date of registration to the date of treatment of progressive CLL or death. Patients who were alive and treatment-free were censored at the date of last follow-up.

Table 1. Patient Characteristics

Characteristic	N=42
Median age (range), y	60 (41-78)
Median time from diagnosis to registration (range), mo	16 (0.7-106)
Male gender	30 (71%)
Median ALC (range), $\times 10^9$/L	33 (10-258)
Rai stage	
0	13 (31%)
I	24 (57%)
II	5 (12%)
ZAP-70 $\geq$20%	12 (29%)
CD38 $\geq$30%	7 (17%)
Unmutated IGHV[a]	6 (18%)
FISH	
(del)13q14.2	27 (64%)
Normal	10 (24%)
Trisomy 12	4 (10%)
(del)11q22	1 (2%)
(del)17p13	0 (0%)

Abbreviations: ALC, absolute lymphocyte count; CD38, cluster of differentiation 38; FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy chain variable region gene; ZAP-70, zeta-chain-associated protein kinase 70.
[a] Although IGHV mutation analysis was performed in all patients, it was noninformative in 9.

RESULTS

Patient Demographics

A total of 37 patients were accrued to the phase 2 portion of the study between September 2007 and October 2010. One patient was deemed ineligible for evaluation because the study drug was not administered correctly during cycle 1. As per protocol, the remaining 36 patients along with the 6 patients in the phase 1 portion of the trial who were treated at the phase 2 dose level were evaluated to determine the tolerability and efficacy of the phase 2 dose level (42 patients). The clinical characteristics of these 42 eligible patients are presented in Table 1. A majority of patients (29 patients; 69%) had Rai stage I to II disease. The majority of patients had favorable prognostic profiles on FISH, ZAP-70, CD38, and IGHV gene mutation analyses at the time of study entry consistent with the eligibility requirements that patients be asymptomatic and have earlier stage disease.

Toxicity and Tolerability

The median overall compliance with the prescribed dose as assessed using pill diaries was 96% (range, 51%-104%). During the 6 months of active treatment, 13 of

Table 2. Side Effects Believed to Be At Least Possibly Related to Therapy

NCI CTCAE Classification	Grade 1	Grade 2	Grade 3
Nausea	23 (55%)	2 (5%)	—
Abdominal pain	9 (21%)	3 (7%)	1 (2%)
Transaminitis	13 (31%)	6 (14%)	1 (2%)
Anorexia	12 (29%)	1 (2%)	—
Diarrhea	19 (45%)	4 (10%)	—
Dyspepsia	11 (26%)	1 (2%)	—
Flatulence	13 (31%)	2 (5%)	—
Fatigue	11 (26%)	3 (7%)	1 (2%)
Hyperglycemia	1 (2%)	—	—

Abbreviation: NCI CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events.

the 42 patients (31%) required a dose reduction. Side effects at least possibly attributed to therapy during the 6 months of active treatment were generally mild, with 18 patients (43%) having a maximum of a grade 2 event and 3 patients (7%) experiencing a grade 3 event (Table 2). Thirty patients completed 6 cycles of active therapy. Twelve patients discontinued therapy early: 9 patients experienced an adverse event and 3 developed PD. As per FDA requirements in all human trials of Polyphenon E, 6 patients were forced to discontinue treatment after experiencing $\geq$ grade 2 transaminitis (5 during the first 6 cycles of treatment and 1 patient during continuation). Changes in the ALC noted in these 6 patients who were forced to

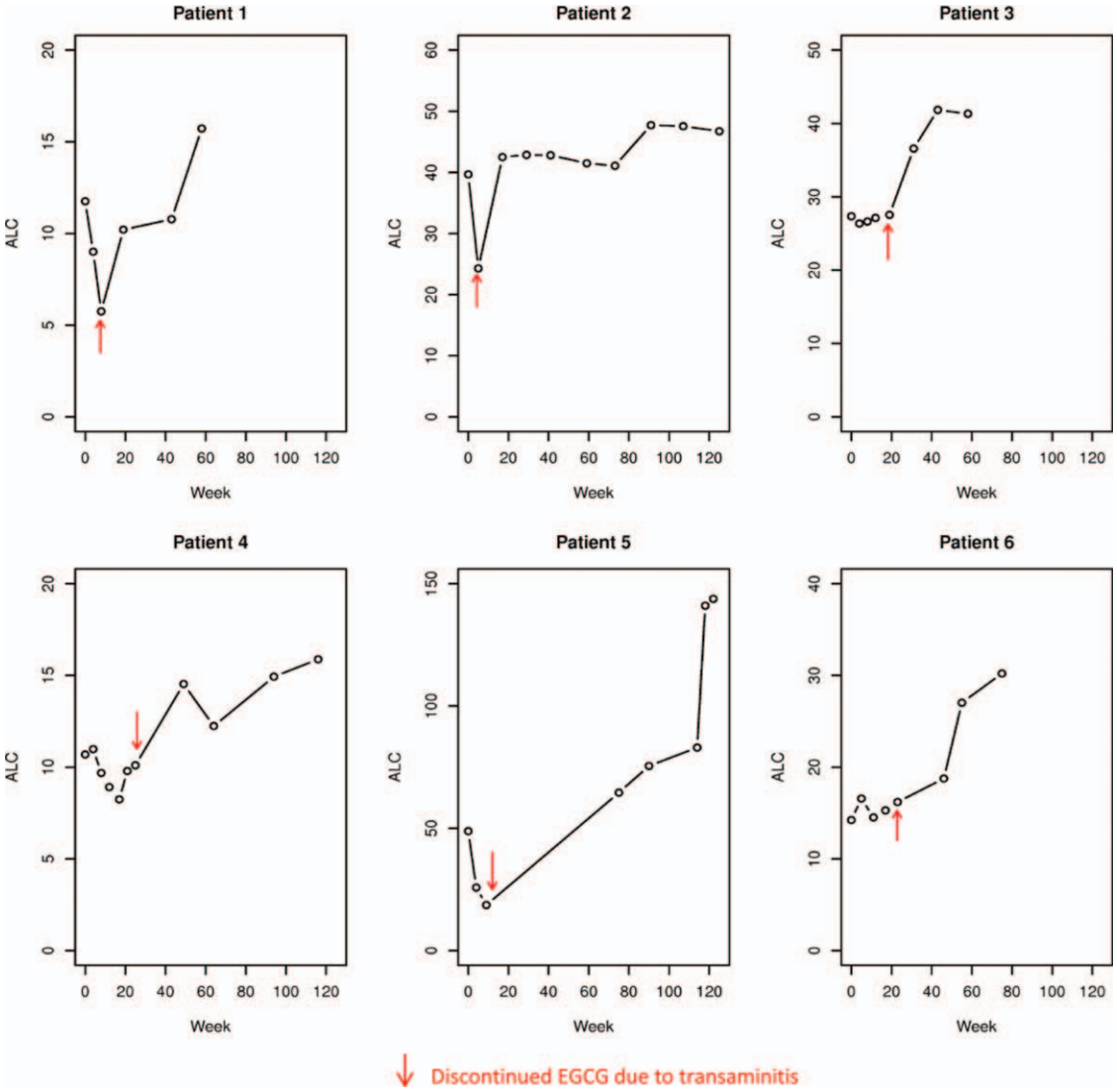

Figure 1. Absolute lymphocyte counts (ALCs) are shown in patients who discontinued therapy because of increased serum transaminase levels. Time 0 indicates the ALC before the initiation of therapy with epigallocatechin gallate (EGCG). Red arrows indicate the ALC at the time EGCG was discontinued due to the increase in serum transaminase levels.

Table 3. Best Response in ALC and Lymph Nodes

Best Reduction in ALC	**No.**	
At least 10% decline	28/42 (67%)	
At least 20% decline	22/42 (52%)	
At least 30% decline	12/42 (29%)	
At least 40% decline	4/42 (10%)	
At least 50% decline	3/42 (7%)	
Best Reduction in Lymphadenopathy	**No.**	
At least 50% reduction in the sum of the products	20/29 (69%)[a]	
PR or Biologic Response	29/42 (69%)	
PR or Biologic Response by Prognostic Parameter	**Percentage With at Least a Biologic Response**	***P***
ZAP-70		
Negative (<20%)	21/30 (70%)	1.0
Positive (≥20%)	8/12 (67%)	
CD38		
Negative (<30%)	25/35 (71%)	.66
Positive (≥30%)	4/7 (57%)	
IGHV		
Mutated	19/27 (70%)	1.0
Unmutated	5/6 (83%)	
FISH		
(del)13q14.2	19/27 (70%)	.84
Normal	7/10 (70%)	
Trisomy 12	2/4 (50%)	

Abbreviations: ALC, absolute lymphocyte count; CD38, cluster of differentiation 38; FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy chain variable region gene; PR, partial remission; ZAP-70, zeta-chain-associated protein kinase 70.
[a] A total of 29 patients had palpable lymphadenopathy at the time of study entry.

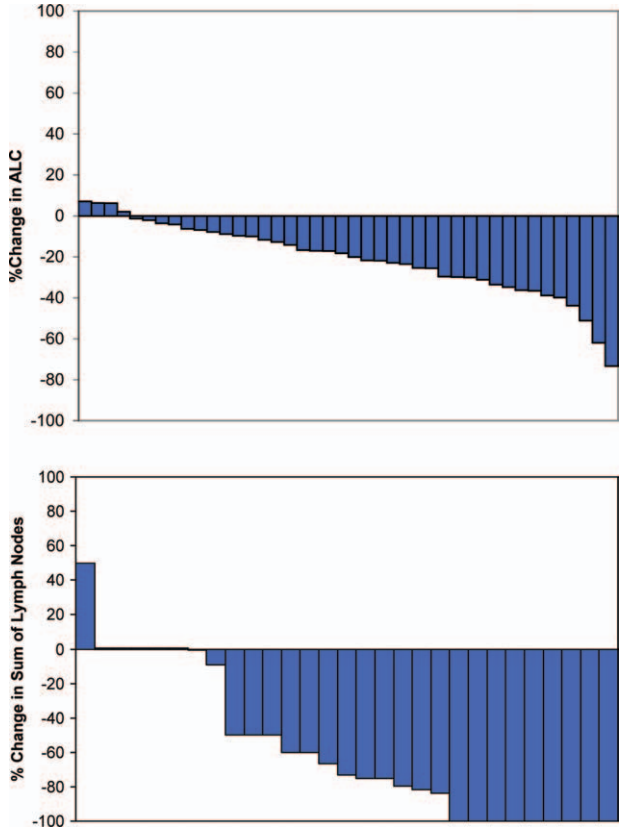

Figure 2. Reductions in the absolute lymphocyte count (ALC) and lymphadenopathy are shown. (*Top*) A waterfall plot of best ALC declines during treatment is shown. (*Bottom*) A waterfall plot of the best reduction in the sum of the products of all lymph node areas is shown.

discontinue treatment after experiencing ≥ grade 2 transaminitis are shown in Figure 1.

Response to Therapy

One patient treated at the phase 2 dose level of the phase 1 trial achieved a partial remission according to the NCI-WG criteria. The majority of patients (67%) had a reduction in the ALC (Table 3) (Fig. 2 Top) that was transient in some patients whereas others experienced a steady, sustained stepwise reduction throughout the 6 months of active therapy. Among the 22 patients (52%) who had a ≥ 20% reduction in their ALC, 13 (31% of the overall study population) had a sustained decrease ≥ 20% persisting for at least 2 months and thus fulfilled the criteria for a biologic response. Among patients who completed 6 cycles of therapy, the ALC at the completion of therapy was below the baseline in 15 patients (47%). Among the 29 patients with palpable adenopathy at the time of enrollment, 20 (69%) experienced at least a 50% reduction in the sum of the products of all palpable lymph node areas at some point during treatment. Collectively, 12 of the 29 patients with Rai stage I/II disease (41%) were downstaged during treatment, including 8 of 24 patients with stage I disease (33%) who experienced a resolution of adenopathy and would be downstaged to Rai stage 0 and 4 patients with stage II disease (100%) (1 of whom was downstaged to Rai stage 0 and 3 of whom were downstaged to Rai stage I) (Fig. 2 Bottom). Among patients with lymphadenopathy at the time of enrollment who completed 6 cycles of therapy, the sum of the products of all lymph node areas at the completion of therapy was below baseline for 13 patients (65%). Overall, 29 patients (69%) fulfilled the NCI-WG criteria for a biologic response.

The percentage of patients with a partial remission or biologic response by each prognostic parameter is

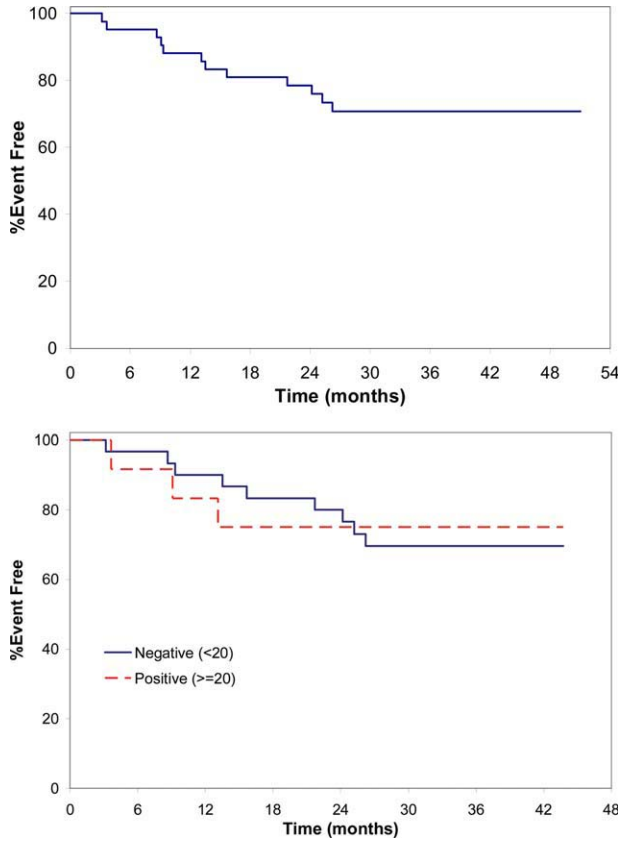

Figure 3. Treatment-free survival is shown from the date of registration. (*Top*) Treatment-free survival for all patients is shown from the date of registration. Time in months is shown on the x-axis. The event-free survival is shown on the y-axis (initiation of treatment of chronic lymphocytic leukemia [CLL] or death were considered events). No deaths had been observed at the time of last follow-up; 8 patients required treatment for progressive CLL. (*Bottom*) Treatment-free survival is shown from the date of registration by zeta-chain–associated protein kinase 70 (ZAP-70) status. The event-free survival of ZAP-70–positive (12 patients) and ZAP-70–negative (30 patients) patients is shown (log-rank $P = .53$).

shown in Table 3. No differences in response were observed based on IGHV, ZAP-70, or CD38 status or on FISH analyses, although the sample size for some comparisons was small.

After a median follow-up of 32 months (range, 21 months-51 months) from the time of registration and a median of 56 months from diagnosis, 12 patients (29%) experienced PD and required treatment for CLL (Fig. 3 Top). The 24-month treatment-free survival rate from registration was 79% (95% confidence interval, 62%-92%) and appeared similar in ZAP-70–positive and ZAP-70–negative patients (Fig. 3 Bottom).

Plasma Polyphenol Levels

The median trough total plasma EGCG level after 1 month of therapy was 188.6 ng/mL, with a wide range of 5.2 ng/mL to 4342 ng/mL (range, 0.001μM-9.56 μM). There was a moderate correlation between plasma EGCG levels and reductions in lymphadenopathy (sum of lymph node products; correlation, 0.44; $P = .02$) at 1 month but not with the reductions in ALC (correlation, 0.18; $P = .28$).

DISCUSSION

Although a majority of patients with CLL have Rai stage 0 to I disease at the time of diagnosis, approximately 70% eventually progress to require treatment and a majority will ultimately die of CLL or CLL-related complications.[26-30] Accordingly, patients with asymptomatic early to intermediate stage CLL represent an appropriate patient population in which to test the ability of nutraceutical agents with a favorable toxicity profile to prevent or delay PD. This approach is conceptually different from the early administration of conventional chemotherapy,[28,31] since it i) does not prematurely expend/exhaust an agent to be used as a treatment later in the course of the disease, ii) is less likely to cause major toxicity (eg, DNA damage) and iii) should not induce chemotherapy resistance. In this phase 2 trial, EGCG in the Polyphenon E preparation was found to be well tolerated at a dose of 2000 mg orally administered twice per day for 6 months in patients with asymptomatic, Rai stage 0 to II CLL. The most severe toxicity was $\leq$ grade 2 for 93% of patients. Although eligibility for this phase 2 trial required a higher baseline ALC than our previous phase 1 trial (ie, 10×10^9/L vs 5×10^9/L), the clinical activity observed was nearly identical to the phase 1 study, with approximately 30% of patients experiencing a sustained decline in their ALC of $\geq$ 20% and approximately 70% of those with lymphadenopathy at the time of study entry experiencing a $\geq$ 50% reduction in the sum of the lymph node products during treatment. Overall, approximately 70% of patients achieved a biologic response, a protocol-specified endpoint developed after discussion and approval by the NCI before the previously published phase 1 study.[18] No difference in the percentage of patients achieving a biologic response was observed based on ZAP-70, CD38, or IGHV gene mutation status. This rate of biologic responses exceeded the protocol-specified decision rule that a biologic response rate of $\geq$ 50% would suggest that EGCG was worthy of further clinical testing.

A higher percentage of patients achieved a biologic response in the phase 2 trial compared with the phase 1 study (approximately 70% vs 55%). This occurred even though the phase 2 trial accrued a higher percentage of patients with Rai stage I to II CLL (69% vs 45%). The

higher biologic response rate could have occurred because the phase 2 trial enrolled exclusively EGCG-naive patients. Alternatively, this finding could suggest that higher doses of EGCG are more effective. This possibility is supported by the higher rate of biologic responses noted in patients being treated with doses $\geq$ 1200 mg twice daily in the phase 1 study compared with those receiving $<$ 1200 mg twice daily (biologic response rate of 76% vs 17%) and the observation that EGCG plasma levels were correlated with reductions in lymphadenopathy in the phase 2 trial.

Although spontaneous regressions occasionally occur in patients with CLL, such remissions are rare (approximately 1% in most series[32-34]). Although fluctuations in ALC and lymphadenopathy can be expected in a subset of patients, the expected pattern for the majority of patients is that of a rising ALC and progressive lymphadenopathy. Among the patients completing 6 months of EGCG, the ALC at the completion of therapy was below baseline in approximately 50% and the sum of the lymph node products at the completion of therapy was below baseline for approximately 65% of patients. The rapid decline in ALC and/or lymphadenopathy observed in a majority of patients after the initiation of therapy with EGCG also strongly suggests that this was a treatment effect. This conclusion is supported by the effect of the cessation of EGCG therapy in patients who developed grade 2 transaminitis. Three of these 6 patients(patients 1, 2, and 5) (Fig. 1) experienced a rapid and substantial (approximately 50%) decline in their ALC within the first 2 months of the initiation of treatment with EGCG followed by an immediate return to near pretreatment levels after discontinuing EGCG.

Although approximately 70% of the patients in the current study had Rai stage I to II CLL, it should be emphasized that the patients who were enrolled were asymptomatic and did not meet criteria to initiate conventional chemotherapy.[22] It is unknown whether the modest clinical effects observed translate into a delay in PD or the need for subsequent chemotherapy. It should also be emphasized that EGCG can in no way be considered a substitute for traditional chemotherapy and/or monoclonal antibody-based treatment once the need for treatment develops.[22] The EGCG-containing preparation used in the current study was a pharmaceutical-grade product with a standardized and verified EGCG dose/content confirmed by the NCI and/or the pharmaceutical manufacturer. Accordingly, it is unknown how the clinical effects reported herein translate to use of over-the-counter, food supplement-grade EGCG-containing products that are not subject to stringent quality control. Furthermore, some animal studies have suggested that a mixture of polyphenols is needed for maximum antitumor effect[35-37] and it is unknown whether the effects observed were related to polyphenols present in Polyphenon E other than EGCG or the specific composition of this EGCG-containing preparation. Although correlative in vitro studies in the current trial did not suggest that EGCG induced resistance to fludarabine or chlorambucil, it is unknown whether these are an accurate approximation of in vivo effects or whether long-term use of EGCG-containing products may influence future sensitivity to conventional chemotherapeutic agents.

There remains great interest on the part of both patients with CLL and their physicians in identifying low-toxicity interventions with the potential to delay PD, particularly for those patients whose molecular prognostic markers (eg, ZAP-70 or IGHV mutation status) predict a higher risk of PD. An effective disease-stabilizing agent must be both efficacious and safe enough for extended use because short-term administration would not be expected to substantially affect the risk of PD over the longer term. EGCG appears to fulfill many of these criteria. Oral EGCG preparations with improved bioavailability are also being developed[38-40] and could be more effective. Ultimately, the ability of EGCG or other nutraceutical compounds to delay PD will need to be determined in a randomized trial.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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