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Bowman Birk Inhibitor Concentrate and Oral Leukoplakia: A Randomized Phase IIb Trial

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Abstract

Oral premalignancy serves as an ideal model for study of chemopreventive agents. Although 13-*cis*-retinoic acid showed reversal of oral premalignancy, toxicity, and reversal of clinical response after cessation of therapy obviated its widespread use. A search for nontoxic agents with cancer preventive activity led us to evaluate Bowman Birk Inhibitor (BBI) formulated as BBI Concentrate (BBIC). We previously reported encouraging results in a phase IIa trial of BBIC in patients with oral leukoplakia with measurable clinical responses and favorable biomarker changes. On the basis of these results, we undertook a randomized, placebo controlled phase IIb trial with patients receiving BBIC or placebo for 6 months, with assessment of clinical response and change in lesion area as primary end point and an intent-to-treat analysis. One hundred and thirty two subjects were randomized; and 89 subjects completed six months on study drug or placebo. Both placebo and BBIC showed a statistically significant decrease in mean lesion area of 17.1% and 20.6%, respectively, and partial or greater clinical responses of 30% and 28% respectively. No significant difference between placebo and study drug arms was observed. Histologic review, review of photographs of lesions, and comparison of serum neu protein and oral mucosal cell protease activity also did not show significant differences between study arms. Probable reasons for these negative results were considered, are discussed, and include a placebo with non-BBIC clinical activity and reduced pharmacokinetic availability of the second batch of BBIC. This experience should be a strong cautionary note to those considering "Green" chemoprevention. *Cancer Prev Res; 6(5); 410–8. ©2013 AACR.*

Introduction

Considerable epidemiologic evidence links dietary habits and incidence of a variety of cancers. Consumption of high levels of soybeans has been associated with decreased incidence of cancer of the breast, colon, and prostate (1–4). Soybeans contain a number of compounds that have potential anticarcinogenic activity including isoflavones, phytic acid, saponins, and several protease inhibitors. Both epidemiologic and experimental data strongly suggest a broad role for protease inhibitors in providing a protective effect against cancer formation, and those with chymotrypsin inhibitory activity have been found to be the most potent (1–5).

The Bowman Birk Inhibitor (BBI) is a serine protease inhibitor isolated from soybeans possessing domains with trypsin and chymotrypsin inhibitory activity (6). *In vitro* and *in vivo* studies show anticarcinogenic activity in a number of animal model systems. BBI Concentrate (BBIC) has the same anticarcinogenic profile as purified BBI, and it has been developed for human trials (5). Both BBI and BBIC are nontoxic, and safety has been reported in a phase I trial of BBIC administered as an oral troche in patients with oral leukoplakia (4). BBIC safety has also been shown in numerous other human trials using BBIC (7).

It has been suggested previously that premalignant human tissues may have elevated levels of proteolytic activity for hydrolysis of the tripeptide fluorescence substrate, butoxycarbonyl-valine-proline-arginine-(7-amino-4-methylcoumarin), which can be used as a biomarker for human cancer prevention studies (8). This proteolytic activity can be inhibited by several anticarcinogenic serine protease inhibitors, including BBI (9). As the abilities of these protease inhibitors to inhibit proteolytic activity

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correlates with their abilities to suppress malignant transformation (9), the hydrolysis activity may be involved in the carcinogenic process, and therefore, could be a candidate biomarker for human cancer prevention studies. Another biomarker potentially useful for human cancer prevention studies is *neu* oncoprotein (10–13). Increased level of neu protein from tumor cells was shown to be correlated with the elevated concentration of neu protein in serum (14, 15). Increased levels of neu protein have been detected as well in patients with various cancers, including cancers of the breast, ovary, prostate, stomach, pancreas, colon, liver, and lung (10, 16, 17). In our phase IIa trial of BBIC modulation, neu protein levels were correlated with changes in protease activity in subjects receiving BBIC (4, 13, 18).

We have previously reported encouraging findings in a phase IIa trial of BBIC in patients with oral leukoplakia (4). Thirty one percent of 32 subjects receiving BBIC for one month showed clinical response. A dose–response relationship in decreased lesion size was observed, and blinded evaluation of lesion photographs confirmed this relationship. Protease activity was found to decrease in individuals with elevated activity at initiation of treatment. These encouraging results led us to proceed with a double-blind randomized placebo controlled trial of BBIC in patients with oral leukoplakia for 6 months. Clinical and histologic response, toxicity, and oral mucosal cell proteolytic activity and Neu levels and serum Neu were evaluated.

Materials and Methods

Design and patient characteristics

This was a 2-armed, double-blind, placebo-controlled, multicenter, phase II-B trial. There were 8 geographically separated performance centers (in California, Massachusetts, New York, and Florida). Subjects had an equal chance of being assigned to the drug or placebo arm. Independent randomization schedules were created for each performance center (so study-arm assignment would not be confounded with geography). For those centers expected to accrue at a faster rate, the randomization schedule incorporated a block size of 4. A block size of 2 was used for centers with lighter accrual goals.

The primary end point was relative percent change in total lesion area after 6 months on study, and the percent of subjects showing a clinical response on that measure. Other end points included change in clinical impression from photographs of lesions, a central pathology review, and changes in buccal-cell and serum Neu protein, and buccal cell protease activity. Early in the study, subjects on the drug arm who showed a partial or complete response at 6 months were allowed to continue treatment for an additional 12 months (total 18 months) with final follow up at 21 months. However, due to the limited supply of drug, the protocol was soon modified to limit treatment to 6 months.

Potential subjects were recruited through radio and print advertising and outreach to dental associations. Eligible participants had to be at least 18 years old and have histologically proven oral leukoplakia and/or erythroplakia capable of being measured bidimensionally (to enable estimates of lesion area). Initially, we required that total lesion area estimated at baseline be at least 100 mm^2, but later this requirement was relaxed to facilitate accrual. Those otherwise meeting the inclusion criteria were excluded for any of the following reasons: (i) use of systemic or topical oral steroids within 3 months, (ii) currently pregnant or lactating, (iii) presence of head-and-neck cancer (including *in situ* disease), or history of such within 2 years, (iv) retinoid or beta-carotene therapy for any reason within 2 years, or beta-carotene capsules of any size within 6 months (patients were allowed up to 2 multivitamins per day), (v) participation in another randomized clinical trial within 6 months. Subjects had to be willing to have photographs taken of the lesion fields, and commit to serial appointments. Premenopausal and perimenopausal women were required to agree to use adequate birth-control methods and have a negative pregnancy test. At the completion of the 4-week run in phase of the study, 75% compliance with administration of drug measured by counting unused drug packets was required for continuation in the study. Written informed consent was obtained for all subjects at initial screening, and the study was approved by the Institutional review boards of the University of California, Irvine (Irvine, CA) and all other performance sites.

Study procedures

After consent and prescreening, potentially eligible subjects underwent a screening oral examination. Buccal mucosal cells were collected, urine specimen obtained, and approximately 15 mL fasting blood drawn at the initial visit. If recent (within 3 months) histologic analysis had not been documented with review of biopsy, 3 mm punch biopsy of representative lesions were conducted after measurement and photodocumentation of lesion(s).

Subjects meeting eligibility criteria underwent a one-month run-in period during which they self-administered the placebo compound. After the run-in period, if compliance with medication was adequate (see above), then baseline examinations were conducted and biospecimens collected. Similar examinations were conducted at 3 months and 6 months on treatment. Lesion biopsy and collection of blood and urine samples were repeated at the 6-month visit. At 3 months off treatment, subjects were evaluated for adverse events and lesion measurements. Subjects were questioned about palatability, side effects, and any adverse events at each visit while on the trial.

Study agent and treatment

For the run-in interval, all patients received placebo, which was Quaker Tortilla Mix–Masa Harina De Maiz. During the run-in period only, 40 mg of citric acid per 3 grams Masa Harina was added for taste. This product was tested for chymotrypsin inhibitory activity and none was measurable. The dose level for placebo was 6 grams per day. Study drug was Bowman-Birk Inhibitor Concentrate (initially produced and supplied by Central Soya Company, but later supplied by the NIH/National Cancer Institute/Division of Cancer Prevention pharmacy). Patients received

daily doses of 600 chymotrypsin inhibitor units of BBIC, administered as 300 chymotrypsin inhibitor units twice a day as 3 grams of BBIC or 3 grams of placebo. Both placebo and drug were provided to subjects as powder in small, zippered, amber, plastic pouches each containing one day's dose. Participants were instructed to use half the material in the morning and the balance in the evening. Patients self-administered, by mixing the appropriate amount of powder with about 20 mL of water, swishing the mixture in the oral cavity for 60 seconds, then swallowing it. Treatment pouches were prepared centrally and sent to performance sites in lots of 100 pouches (3-month supply) per participant, renewed upon need.

Assessment of clinical response

The primary clinical response measure for each subject at each time point was estimated relative percent change in total lesion area, with primary interest being the comparison of baseline measures to those at 6 months on treatment. Clinical examiners recorded the estimated extent of the longest axis of each lesion and the extent perpendicular to the longest axis, in mm, at each time point for each subject. Clinicians made reference to previous exams to ensure all lesions were measured, recording values of zero for lesions that had resolved, and to document new lesions. After data entry, individual lesion areas were estimated by the product of length and width, and summed across lesions within each subject, by time point. Relative percent change in total lesion area was defined as 100 times (area posttreatment minus area pretreatment) all divided by pretreatment area. Category of clinical response was based on the magnitude of relative percent change in total lesion area. A complete response (CR) was declared if the relative percent change in total lesion area was minus 100 percent. A partial response (PR) was a relative percent decrease in total lesion area of 50% or more, without being a CR. Disease progression was a relative percent increase in total lesion area of at least 50%. Remaining cases were declared to be stable disease.

A secondary clinical response measure was based on blinded, comparative judgments of pairs of photographs of the same lesion at baseline and 6 months on study. Study staff assembled all available pictures of lesions from pretreatment (screening or randomization) and the 6 month time points, by subject and lesion. Pictures at randomization were preferred over those at screening, but the latter were used if the former were absent. Any picture from one time point without a mate at the other time point was set aside. (Note: lesions first appearing during the course of treatment had no mate from pretreatment exams.) If a subject had multiple picture pairs for the same lesion, then one pair was chosen at random and the others set aside. In case of multiple pictures for a lesion at one time point, then one was picked at random for use and the others set aside. If there were picture pairs for more than one lesion for a particular subject, then one pair was selected at random for use and the others set aside. Thus, each subject having photos of the same lesion (or appropriate anatomic area, if the lesion had resolved) contributed exactly one picture pair to the task materials.

Picture pairs were arranged into a photo album, with one pair per page, one photo above the other. Picture pairs were assigned to album page at random, such that, both study performance center and treatment arm were balanced across the presentation sequence, as was whether the pretreatment picture or the 6-month picture was in the superior position. Five physicians experienced with evaluation of oral mucosal tissue abnormalities, but blinded to study arm and time point, independently compared the pictures in each pair using a 7-point scale. The scale ranged from, "top photo shows a complete response relative to the bottom photo," through, "the same degree of disease is shown by top photo and bottom photo," to "bottom photo shows a complete response relative to the top photo." Raw scores were transformed to account for relative position of the earlier and later photo, and averaged across the 5 reviewers. Final scores ranged from one, denoting a CR at 6 months, to 4, which indicated no change, through 7, which indicated that the 6-month photo depicted a much worse situation than the pretreatment photo.

Pathologic assessment

A single, experienced pathologist reviewed pre- and posttreatment pairs of tissue specimens. The reviewer was blinded to study-arm assignment (drug or placebo), but not to time point of specimen. For each specimen, the reviewer marked a continuum to indicate degree of tissue abnormality. The continuum was 140 mm long, and anchored by the word 'Normal' on the left and 'Malignant' on the right. The distance from the left edge of the continuum to the reviewer's mark, in mm, was determined. For analyses, a score was formed by subtracting the pretreatment value from the 6-month value. Thus, a retreat from 'Malignancy' over time produces a negative score, a score of zero denotes no change, and a positive score denotes a worsening situation.

The central pathologist also made a direct comparison of pre- and posttreatment specimens, marking a 170 mm continuum anchored on the left by "Posttreatment shows no dysplasia in comparison with pretreatment," and on the right by, "Posttreatment shows greater dysplasia than pretreatment." The center of the continuum was labeled, "Pretreatment and posttreatment show no difference." The reviewer's mark was coded as the distance from the left edge, in millimeters. On this measure, low scores denote improvement over time, a score of 85 denotes no change, and a value higher than 85 indicates histologic worsening.

Intermediate marker endpoints

Titer information was developed for Buccal-Cell Neu protein (ng/mg), Serum Neu protein (ng/ml), and Protease Activity (delta RFU/min/μg). Laboratory and assay methods were the same as described by Wan and colleagues (13). Titer amounts by subject number and date were forwarded to the statistician, who consolidated the information. For all 3 intermediate marker endpoints (IME), multiple readings for

the same subject at the same time point were averaged. IME data are reported as the relative percent change from pretreatment to 6 months (post minus pre, all divided by pre).

Statistical analyses and data management

Power estimates for our phase IIB study were informed by the dose response observed in our preceding, single-arm, phase IIA trial in which study response rate (PR+CR) at the lowest dose (200 CIU) was 12.5% (1/8 participants). We used this figure to anticipate response in the placebo arm of the phase IIB effort. Similarly, we used the phase IIA figure of 36.4% (4/11) responding (PR+CR) at the 533 CIU dose to anticipate the response rate of the treatment arm in the phase IIB effort. Thus, the phase IIB trial was powered to contrast response rates of about 12.5% and 36%. In both the phase IIA and IIB trials, our criterion for a partial response was a reduction of estimated lesion area of at least 50%.

Analyses were conducted using SAS/STAT software (19). Raw data were entered into a central database (Microsoft Access) by staff of the Biostatistics Shared Resource of the University of California Irvine Chao Family Comprehensive Cancer Center. Data on lesion size and judgments of photos and pathology specimens were formed into SAS data sets that were subject to 100% verification against the case-report forms by the study statistician. Results of IME assays were entered into spreadsheets by wet-lab staff, which the statistician converted to SAS data sets, which were then verified against the original spreadsheets. Data on host factors were extracted from the central database. Statistical tests of proportions were by Fisher's exact test (20). Because measures of relative percent change profoundly violate normality, as did estimates of total lesion area and the pathology judgments, comparisons of continuous measures between groups were by Wilcoxon 2 sample test (20). The signed-rank test was used to evaluate the null hypothesis that measures of change were centered on zero (20). Confidence intervals about medians are based on order statistics. Correlations among relative percent change scores were by Spearman rank correlation (21). No adjustment was made for multiple testing or for any correlation among the various dependent measures.

Results

Participants

A total of 513 people were screened for participation across all 8 performance sites, of which 188 (37%) were ineligible and 325 were consented. The date of first randomization was May 1999 and the last in September 2009. Of those screened, 118 (23%) did not complete the run-in period. Of those consented, 157 (48%) completed the run-in period but 25 declined to continue, resulting in 132 randomized patients. Sixty-seven patients were randomized to the drug arm, whereas 65 were assigned to placebo. The difference in numbers assigned to the 2 arms did not exceed one at any performance site. Of the 132 randomized, 89 participants (67.4%) had lesion sizes recorded at both randomization and 6 months, and so were evaluable on the primary end points. Twenty-one participants had lesion sizes recorded at randomization, but not at 6 months. Furthermore, 22 had no lesion measurements recorded at randomization (the majority from a remote performance site, with high turnover in study personnel).

The 89 subjects who were evaluable on the primary end point at 6 months seem fairly representative of the 132 who were randomized (Table 1). Importantly, the proportion of those randomized who contributed data at 6 months did not differ between study arms [drug arm 64% (43/67), placebo arm 71% (46/65); $P > 0.46$] nor did that proportion differ by gender ($P > 0.44$) or major race/ethnic group ($P > 0.57$). About 36% (48/132) of those randomized reported use of beer, wine, or liquor, whereas 42% (56/132) did not answer questions on alcohol consumption. Proportions reporting, not reporting, or denying alcohol use did not differ between those who did and did not contribute data on the primary end point at 6 months ($P > 0.39$). About 21% of those randomized (28/132) reported using tobacco (viz., cigarettes, cigars, pipe, oral use), whereas 50% (66/132) did not answer questions on tobacco use. Proportions reporting, not reporting, or denying tobacco use did not differ between those who did and did not contribute data on the primary end point at 6 months ($P > 0.25$). Estimates of total lesion area at randomization did not differ between those with lesion measurements only at randomization ($n = 21$, mean 530.7 mm^2, median 380 mm^2, range 19–1976 mm^2) and those evaluable on the primary end point at 6 months ($n = 89$, mean 502.6 mm^2, median 310 mm^2, range 48–3500 mm^2; $P > 0.70$).

Table 1 also summarizes measures describing the patients evaluable on the primary end point at 6 months, overall and by study arm. Fifty-seven men and 32 women contributed data on relative percent change in total lesion area (the primary end point) at 6 months. The proportion that were male did not differ across study arm ($P > 0.99$), nor did the distribution of age ($P > 0.15$). About 78% (69/89) were White, and the proportion of Whites did not differ across study arm ($P > 0.99$). The proportions reporting, not reporting, or denying alcohol or tobacco use, did not differ between study arms (alcohol $P > 0.82$; tobacco $P > 0.29$). Estimated total lesion area at randomization did not differ between study arms ($P > 0.64$).

Clinical response

Table 2 shows the number and proportion showing various degrees of clinical response, by study arm (degree of clinical response is defined in the Materials and Methods section above). Both the number and proportion of participants in each category of clinical response are similar across study arms ($P > 0.94$). About 28% of those in the drug arm achieved a partial response or better (12/43) while that figure for the placebo arm was 30% (14/46), which are not statistically different ($P > 0.81$). The 2 arms produced similar rates of disease progression: 9% in the drug arm (4/43) and 13% in the placebo arm (6/46; $P > 0.74$). Thus the data in Table 2 show no evidence that the treatment produced a shift in the distribution of clinical response, relative to the placebo arm.

Table 1. Patient characteristics by study arm (for those evaluable on relative percent change in total lesion area at six months)

Characteristic	Overall	Drug	Placebo	Difference between arms
Number of participants	89	43	46	% dropouts NS
Age				
Mean	60.7	58.7	62.6	NS
Median	61.8	59.7	63.0	
Range	29–82	29:82	35:82	
Sex (%male)	64% (57/89)	65% (28/43)	63% (29/46)	NS
Race/Ethnicity (%White)	78% (69/89)	33/43	36/46	NS
Alcohol Use (self report)				
Yes	35/89	16/43	19/46	NS
No	20/89	11/43	9/46	
Unknown	34/89	16/43	18/46	
Tobacco use (self report)				
Yes	20/89	12/43	8/46	NS
No	29/89	11/43	18/46	
Unknown	40/89	20/43	20/46	
Total lesion area at randomization				
Mean	502.6	492.4	512.1	NS
Median	310	300	317.5	
Range	48–3500	48–3500	55–2550	

Figure 1 shows the distribution of relative percent change in total lesion area after 6 months on treatment, by study arm, and Table 3 gives corresponding summary measures of these distributions. As the figure shows, the bulk of scores, ignoring study arm, fall below zero (sign-rank $\underline{P} < 0.0002$) implying that lesion area was reduced in both groups. Per Table 3, the median relative percent change across study arms was about a 26% reduction. The distributions by study arm seem reasonably coincident, with no sign of a difference in location ($P > 0.75$) or spread ($F(45,42) = 1.60$, $P > 0.12$).

Clinical impression from photographs confirmed these assessments (Supplemental 1). No significant adverse events related to drug or placebo administration were observed during the trial (details in Supplemental 2). This assessment included the 10 patients treated with BBIC or placebo for up to 18 months in the trial. Medication was tolerated, with some subjects expressing palatability issues. The distribution of adverse events in the 2 arms was not different. Pre and post levels of cholesterol and tryglycerides was similar in the 2 groups (Supplemental 3).

Table 2. Number and percent of subjects by category of clinical response at six months, by study arm

Clinical response	Drug	Placebo
CR	2 (4.6%)	2 (4.4%)
PR	10 (23.3%)	12 (26.1%)
Stable disease	27 (62.8%)	26 (56.5%)
Disease progression	4 (9.3%)	6 (13%)
Total	43 (100%)	46 (100%)

NOTE: CR: 100% reduction in total lesion areas. PR: atleast a 50% reduction, but not complete resolution, of total lesion areas. Stable disease: disease progression: FET $P > 0.90$

Central pathologic review

The central pathologist reviewed 98 pairs of slides, a small percentage of which were re-cuts substituted for original material that could not be evaluated. Three subjects had materials for multiple lesions, but for analysis, we randomly selected just one lesion in each case. Each participant contributed just one lesion to the analysis. One subject was dropped because the only available postrandomization specimen was from the 3-month time point. The materials for an additional 4 subjects were deemed inadequate for review, and no re-cuts were available. Thus final analysis was based on 88 subjects, representing 67% of those randomized (88/132). This proportion did not differ statistically across study arm (drug: 41/67; placebo: 47/65; $P > 0.19$).

Table 4 summarizes the difference in rated degree of malignancy between randomization and 6-month specimen. Positive values indicate histologic worsening, whereas negative scores denote improvement over the 6-month study period. Across study arms, the location of these scores is not statistically different from zero (sign-rank, $P > 0.46$), and, consistent with inspection of the table, there is no

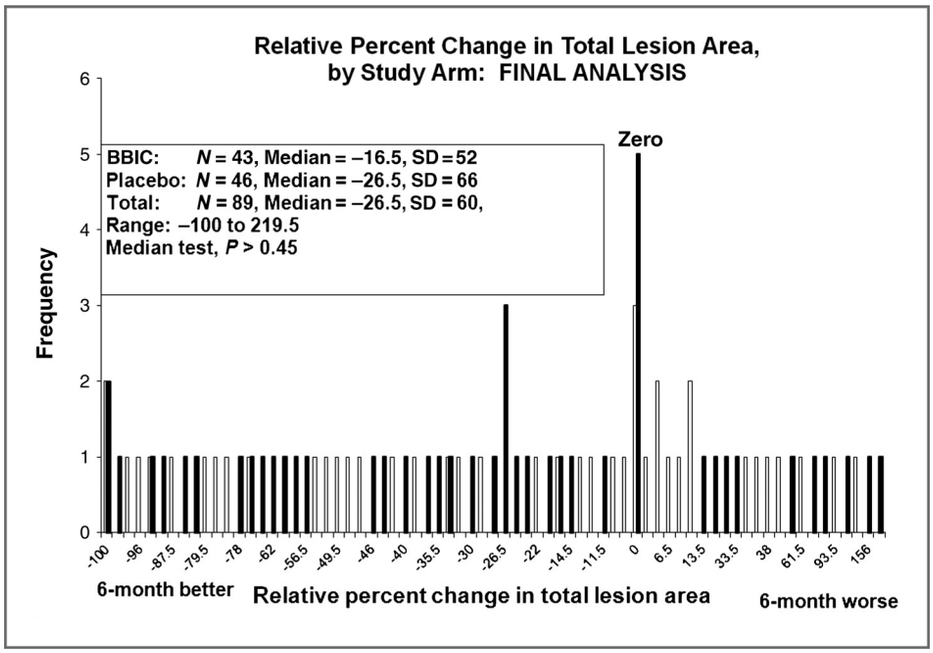

Figure 1. Relative Percent Change in Total Lesion Area, by Study Arm: FINAL ANALYSIS (Note: BBIC-grey bars, Placebo-solid bars)

difference between groups ($P > 0.88$). A similar result was obtained from the pathologist's direct comparison of the specimens. The median score overall and for each study arm was that point indicating no change, and of course there is no significant difference between the 2 arms ($P > 0.68$: data not shown).

Intermediate markers

Table 5 shows relative percent change over 6 months on-study in buccal-cell Neu protein and protease activity, and serum-Neu protein, overall and for each study arm. Necessary specimens and evaluable results for these assays were available for 45, 45, and 41 subjects, respectively. Pooled across study arm, the relative percent change in protease activity was statistically elevated above zero (sign-rank, $P < 0.05$, $n = 45$). However, neither study arm showed this effect when considered separately (placebo: $P > 0.07$; drug: $P > 0.30$). Relative percent change in the remaining 2 markers was not statistically different from zero, either overall (ps > 0.18) or by study arm (ps > 0.30). None of the 3 measures showed a statistically significant difference between placebo and drug arms (ps > 0.32). When study arm was ignored, none of these 3 measures showed a significant correlation with relative percent change in total lesion area (buccal-cell Neu: rank correlation 0.11, $P > 0.45$, $N = 45$; protease activity: rank correlation 0.92, $P > 0.88$, $N = 45$; serum Neu: rank correlation 0.07, $P > 0.66$, $N = 41$).

Table 3. Summary of relative percent change in total lesion area from baseline to six months on study, by study arm

Study arm	*N*	Mean (SD)	Median	Smallest	Largest
Drug	43	−20.6 (52.4)	−16.5	−100.0	+123.5
Placebo	46	−17.1 (66.4)	−26.5	−100.0	+219.5
TOTAL	89	−18.8 (59.7)	−26.5	−100.0	+219.5

NOTE: Wilcoxon 2-sample test, $P > 0.75$.

Discussion

In this phase IIb clinical trial of BBIC, no significant difference between placebo and treatment arms was identified for clinical response, change in lesion area, histologic grade, or biomarkers studied. Interestingly, both the treatment and placebo group showed statistically significant decreases in total lesion area (Table 3), and the 2 groups showed PR + CR rates of 27.9% and 30.5%, respectively (Table 2). No significant difference in photographic review of lesions was identified between study groups, but the placebo group showed slight improvement following treatment. Oral leukoplakia has a waxing and waning clinical course, and spontaneous improvement or disappearance has been reported in more than 10% of cases (22, 23). However, the approximate 30% response rate raises the possibility that there was clinical activity in the placebo used in the trial.

In designing the study, considerable attention was directed at placebo selection. It was very difficult to identify a compound that would be inert, palatable, and have similar consistency and physical properties similar to those of BBIC. Ultimately, a corn-based product was selected, and Masa Harina was used. Although no chymotrypsin inhibitory activity was measurable, this product contains several vitamins, antioxidants, protease inhibitors, and supplemental nutrients, and is processed by lime cooking, which

Table 4. Summary measures of the difference in rating of degree of malignancy of tissue from pretreatment to six months on study, by study arm

Study arm	*N*	Mean (SD)	Median	Smallest	Largest
Drug	41	1.2 (23.7)	0	−86	71
Placebo	47	3.6 (15.4)	0	−20	59
TOTAL	88	2.5 (19.6)	0	−86	71

NOTE: Negative scores indicate a change toward normal tissue. Positive scores indicate a change toward malignancy. Values of zero indicate no change.

alters levels of phytochemicals and phenolic compounds, such as ferulic acid (24). The levels of calcium and ferulic acid were sufficiently high in the placebo that cancer preventive activity may have been present; in addition, several other nutrients were present in the placebo at micromolar concentrations that have been shown to have radioprotective or cancer preventive activity in other studies (as described in Supplemental 4). These properties raise the issue of the placebo having had an unexpected clinical effect. Similar concerns about a placebo effect have been raised in a trial of the COX inhibitor ketorolac as an oral rinse in oropharyngeal leukoplakia (25), suggesting that the placebo oral rinse, which contains alcohol, and improved oral hygiene may contribute to a beneficial overall effect on oral inflammatory processes. In studies of other oral inflammatory processes, higher than expected response rates were attributed to increased frequency of oral rinsing (26, 27). In total, this report indicates that future trials of chemoprevention agents in oral leukoplakia need to carefully consider whether a no treatment arm should be part of the trial and/or a much larger trial should be done. However, with a 30% placebo effect, trials become prohibitively large (see Supplemental 6 for discussion).

The potential activity of the placebo raises concerns about the overall result. A number of natural compounds have potential chemopreventive activity, and if these compounds are biologically active, they will mask effects of the study drug tested in placebo-controlled trials. Even limited activity could mask the clinical effect of the study drug, and require larger sample sizes to distinguish statistically significant differences in clinical effect. Given the difficulty of accruing subjects in chemoprevention studies, this will make these studies even more expensive and prolonged. This dilemma has raised the question of whether alternative study designs would be more effective. For example, a study design in which the patient serves as her/his own control with serial periods on or off study drug may eliminate issues related to placebo activity.

To further complicate the evaluation, there seems to have been a loss in drug potency over time. An interim analysis was conducted approximately half way through the study. Preliminary blinded analysis indicated a modest but promising difference in treatment response between the 2 study arms (Supplemental 5). On the basis of these encouraging results, the trial was continued and completed. Analysis of the subjects accrued during the second half of the trial showed no difference between the 2 arms of the study. These results suggest that patients treated with BBIC in the earlier years of the trial exhibited better responses, in terms of effects on size of the oral leukoplakia lesions as well as the intermediate marker end points evaluated, than those treated with BBIC in the later trial years, (as detailed in Supplemental 5). It is hypothesized that the apparent loss of potency could have been caused by the BBI storage conditions during this long time period. The directions that came with packages of BBIC initially prepared by Central Soya Company (and dispensed during the early years of the oral leukoplakia trial) indicated that BBIC should not be refrigerated or frozen, as it was known at an early stage of BBIC development that storage under refrigerated or frozen conditions greatly diminished the ability of BBIC to inhibit the malignant transformation of cells

Table 5. Median relative percent change in biomarkers, by study arm

ARM:	Overall		Placebo		Drug	
MARKER	Median	95% CI	Median	95% CI	Median	95%CI
Buccal-Cell	−8.9	−24.5	−4.2	−33.6	−10.1	−52.3
Neu	N = 45	+18.5	N = 24	+36.7	N = 21	+24.6
Protease	+16.2	−10.0	+17.2	−10.3	+15.7	−23.8
Activity	N = 45	+27.0	N = 24	+30.9	N = 21	+74.9
Serum	−4.1	−11.3	−8.1	−15.3	−3.9	−13.5
Neu	N = 41	+0.7	N = 22	+6.6	N = 19	+8.1

in vitro (28). Retrospectively, it was discovered that a different batch of BBIC (termed the new formulation of BBIC), maintained by the same pharmacy that dispensed BBIC during the later years of the oral leukoplakia trial, was kept at both refrigerated and frozen temperatures. Analysis of the bioavailability of the original and new formulations of BBIC in human subjects showed an approximately 60% reduction in bioavailability of the new formulation, compared with the original formulation, of BBIC (unpublished results, made available to the reviewers). As the new formulation of BBIC was maintained under both refrigerated and/or frozen conditions by the same pharmacy that provided the BBIC for the oral leukoplakia trial, it is conceivable that storage of BBIC in a refrigerated/frozen state could have decreased the clinical activity of the drug during the later years of the oral leukoplakia trial. The reduced bioavailability of the second BBIC product also raises concerns as our phase IIA trial showed a clear dose–response effect (4) and a 40% bioavailability would be below the effectiveness of the chosen dose in the current phase IIB trial. Another difficulty encountered was the long time duration between trial initiation and completion; patients with oral leukoplakia were actually treated with BBIC over a period of approximately 11 years (May 1999 to March, 2010).

In conclusion, this phase IIb randomized placebo-controlled trial did not show greater efficacy for the BBIC compared with placebo in patients with oral leukoplakia. This experience should be a strong cautionary note to those considering "Green" chemoprevention (29).

Disclosure of Potential Conflicts of Interest

F.L. Meyskens has ownership interest (including patents) in Cancer Prevention pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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References

1. Messina M, Barnes S. The role of soy products in reducing risk of cancer. J Natl Cancer Inst 1991;83:541–6.
2. Messina MJ, Persky V, Setchell KD, Barnes S. Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. Nutr Cancer 1994;21:113–31.
3. Fournier DB, Erdman JW, Gordon GB. Soy, its components, and cancer prevention: a review of the *in vitro*, animal, and human data. Cancer Epidemiol Biomarkers Prev 1998;7:1055–65.
4. Armstrong WB, Kennedy AR, Wan XS, Taylor TH, Nguyen QA, Jensen J, et al. Clinical modulation of oral leukoplakia and protease activity by Bowman-Birk inhibitor concentrate in a phase IIa chemoprevention trial. Clin Cancer Res 2000;6:4684–91.
5. Kennedy AR. Chemopreventive agents: protease inhibitors. Pharmacol Ther 1998;78:167–209.
6. Birk Y. The Bowman-Birk inhibitor. Trypsin- and chymotrypsin-inhibitor from soybeans. Int J Pept Protein Res 1985;25:113–31.
7. Kennedy AR. The status of human trials utilizing Bowman-Birk Inhibitor concentrate from soybeans. In: Soy in Health and Disease Prevention, edited by Michichiro Sugano, Taylor & Francis, Boca Raton, FL:2005;207–33.
8. Manzone H, Billings PC, Cummings WN, Feldman R, Clark LC, Odell CS, et al. Levels of proteolytic activities as intermediate marker endpoints in oral carcinogenesis. Cancer Epidemiol Biomarkers Prev 1995;4:521–7.
9. Billings PC, Carew JA, Keller-McGandy CE, Goldberg AL, Kennedy AR. A serine protease activity in C3H/10T1/2 cells that is inhibited by anticarcinogenic protease inhibitors. Proc Natl Acad Sci U S A 1987;84:4801–5.
10. Brandt-Rauf PW. The c-erbB transmembrane growth factor receptors as serum biomarkers in human cancer studies. Mutat Res 1995;333:203–8.
11. Smit EF, Groen HJ, Splinter TA, Ebels T, Postmus PE. New prognostic factors in resectable non-small cell lung cancer. Thorax 1996;51:638–46.
12. Meden H, Kuhn W. Overexpression of the oncogene c-erbB-2 (HER2/neu) in ovarian cancer: a new prognostic factor. Eur J Obstet Gynecol Reprod Biol 1997;71:173–9.
13. Wan XS, Meyskens FL Jr, Armstrong WB, Taylor TH, Kennedy AR. Relationship between protease activity and neu oncogene expression in patients with oral leukoplakia treated with the Bowman Birk Inhibitor. Cancer Epidemiol Biomarkers Prev 1999;8:601–8.
14. Leitzel K, Teramoto Y, Sampson E, Mauceri J, Langton BC, Demers L, et al. Elevated soluble c-erbB-2 antigen levels in the serum and effusions of a proportion of breast cancer patients. J Clin Oncol 1992;10:1436–43.
15. Wu JT, Astill ME, Gagon SD, Bryson L. Measurement of c-erbB-2 proteins in sera from patients with carcinomas and in breast tumor tissue cytosols: correlation with serum tumor markers and membrane-bound oncoprotein. J Clin Lab Anal 1995;9:151–65.
16. Brandt-Rauf PW, Pincus MR, Carney WP. The c-erbB-2 protein in oncogenesis: molecular structure to molecular epidemiology. Crit Rev Oncog 1994;5:313–29.
17. Tokunaga A, Onda M, Okuda T, Teramoto T, Fujita I, Mizutani T, et al. Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric cancer. Cancer 1995;75(6 Suppl):1418–25.

18. Armstrong WB, Wan XS, Kennedy AR, Taylor TH, Meyskens FL Jr. Development of the Bowman-Birk inhibitor for oral cancer chemoprevention and analysis of Neu immunohistochemical staining intensity with Bowman-Birk inhibitor concentrate treatment. Laryngoscope 2003;113:1687–702.
19. SAS OnlineDoc® 9.1.3. 2007 September 22, 2012]; Available from: http://support.sas.com/onlinedoc/913/docMainpage.jsp.
20. Bradley JV. Distribution-free statistical tests. 1968, Englewood Cliffs, NJ: Prentice-Hall. xii, 388.
21. Dawson B, Trapp RG. Basic & clinical biostatistics. 4th ed; 2004, New York, NY: Lange Medical Books/McGraw-Hill, Medical Pub. Division. x, p. 438.
22. Mincer HH, Coleman SA, Hopkins KP. Observations on the clinical characteristics of oral lesions showing histologic epithelial dysplasia. Oral Surg Oral Med Oral Pathol 1972;33:389–99.
23. Pindborg JJ, Daftary DK, Mehta FS. A follow-up study of sixty-one oral dysplastic precancerous lesions in Indian villagers. Oral Surg Oral Med Oral Pathol 1977;43:383–90.
24. de la Parra C, Saldivar SO, Liu RH. Effect of processing on the phytochemical profiles and antioxidant activity of corn for production of masa, tortillas, and tortilla chips. J Agric Food Chem 2007;55: 4177–83.
25. Mushine JL, Atkinson JC, Greer RO, Papadimitrakopoulou VA, Van Waes C, Rudy S, et al. Randomized, double blind, placebo-controlled phase IIB trial of the cyclooxygenase inhibitor ketorolac as an oral rinse in oropharyngeal leukoplakia. Clin Cancer Res 2004; 10:1565–73.
26. Dodd MJ, Larson PJ, Dibble SL, Miaskowski C, Greenspanc D, MacPhail L, et al. Randomized clincial trial of chlorhexidine versus placebo for prevention of oral mucositis in patients receiving chemotherapy. Oncol Nurs Forum 1996;23:921–7.
27. Meiller TF, Kutcher MJ, Overholser CD, Niehaus C, DePaola LG, Siegel MA. Effect of an antimicrobial mouthrinse on recurrent aphthous ulcerations. Oral Surg Oral Med Oral Pathol 1991;72:425–9.
28. Kennedy AR, Szuhaj BF, Newberne PM, Billings PC. Preparation and production of a cancer chemopreventive agent, Bowman-Birk inhibitor concentrate. Nutr Cancer 1993;19:281–302.
29. Fahey JW, Talalay P, Kensler TW. Notes from the field: "green" chemoprevention as frugal medicine. Cancer Prev Res 2012; 5:179–88.

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