Soy Isoflavones in the Treatment of Prostate Cancer

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Abstract: *Epidemiological studies suggest an inverse association between soy intake and prostate cancer (Pca) risk. We have previously observed that soy isoflavone genistein induces apoptosis and inhibits growth of both androgen-sensitive and androgen-independent Pca cells in vitro. To determine the clinical effects of soy isoflavones on Pca we conducted a pilot study in patients with Pca who had rising serum prostate-specific antigen (PSA) levels. Patients with Pca were enrolled in the study if they had either newly diagnosed and untreated disease under watchful waiting with rising PSA (group I) or had increasing serum PSA following local therapy (group II) or while receiving hormone therapy (group III). The study intervention consisted of 100 mg of soy isoflavone (Novasoy®) taken by mouth twice daily for a minimum of 3 or maximum of 6 mo. Forty-one patients were enrolled (4 in group I, 18 in group II, and 19 in group III) and had a median PSA level of 13.3 ng/ml. Thirty-nine patients could be assessed for response. Soy isoflavone supplementation was given for a median of 5.5 (range 0.8–6) mo per patient. Although there were no sustained decreases in PSA qualifying for a complete or partial response, stabilization of the PSA occurred in 83% of patients in hormone-sensitive (group II) and 35% of hormone-refractory (group III) patients. There was a decrease in the rate of the rise of serum PSA in the whole group (*$P = 0.01$*) with rates of rise decreasing from 14 to 6% in group II (*$P = 0.21$*) and from 31 to 9% in group III (*$P = 0.05$*) following the soy isoflavone intervention. Serum genistein and daidzein levels increased during supplementation from 0.11 to 0.65 μM (*$P = 0.00002$*) and from 0.11 to 0.51 μM (*$P = 0.00001$*), respectively. No significant changes were observed in serum levels of testosterone, IGF-1, IGFBP-3, or 5-OHmdU. These data suggest that soy isoflavones may benefit some patients with Pca.*

Introduction

Epidemiologic studies have shown an inverse association between soy consumption and prostate cancer (Pca) risk (1–4). Isoflavones have been suggested as the principal chemical constituents responsible for the potential preventive effect of soy against Pca (5). In some Asian countries with high soy consumption, the incidence of latent and small prostate carcinomas is the same as in Western countries, whereas the mortality from clinically diagnosed Pca is lower (6), suggesting that soy isoflavones may also inhibit the progression of Pca. A variety of possible mechanisms have been proposed for the activity of soy isoflavones in Pca, which include estrogen-like effects (7), prevention of oxidative DNA damage (8,9), reduction in cancer cell proliferation (10), inhibition of angiogenesis (11), modulation of steroid-metabolizing enzymes (12), tyrosine kinase (13) and topoisomerase II (14), and effects on signal transduction molecules (15).

We have previously observed that soy isoflavone genistein inhibits Pca cell growth in culture in a dose-dependent manner accompanied by a G2/M cell cycle arrest (15). Genistein-induced inhibition of cell growth was associated with down-regulation of cyclin B, up-regulation of the $p21^{WAF1}$, and induction of apoptosis. We have also observed that oxidative stress (TNF-α or H_2O_2) –induced activation of transcription factor NF-κB can be abrogated by genistein in Pca cell lines (16) and by soy isoflavone supplementation in normal human volunteers (17). These data provided the rationale for investigating the in vivo effects of genistein in patients with Pca. Because serum prostate-specific antigen (PSA) is a well-established marker that correlates with Pca progression, we conducted a pilot study to investigate the effect of soy isoflavone supplementation in patients with rising serum PSA levels.

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Methods

This pilot trial was designed with broad eligibility criteria to allow study entry for any patient with Pca who had a rising serum PSA to primarily detect the effect of soy isoflavones on serum PSA. Patients had to have histologically proven Pca with a rising PSA and one of the following criteria: 1) untreated disease in patients electing watchful waiting that is deemed appropriate by the managing physician (group I); 2) patients whose only indication of relapse was rising PSA after having had radical prostatectomy or radiation therapy (group II); or 3) patients relapsing after having received hormone therapy, that is, those with androgen-independent PSA progression with or without metastasis following hormonal therapy and anti-androgen withdrawal (4 wk for flutamide and 6 wk for bicalutamide) when applicable (group III). Rising PSA was defined as three successive levels at least 2 wk apart with a rising trend or PSA of ≥10 ng/ml at two successive evaluations at least 2 wk apart. LHRH-agonist therapy was continued, but patients had to be off any other hormonal therapy for a minimum of 4 wk and as specified previously for anti-androgen therapy. Patients who received prior chemotherapy were eligible but had to be off chemotherapy for 4 wk and fulfill the rising PSA criterion. Other concomitant therapy for Pca was not allowed during the soy isoflavone intervention. Patients taking other supplements, such as vitamin E, lycopene, or selenium, were not eligible to participate. Patients were allowed to take a single standard-dose multivitamin daily if they wished. Patients had to have a life expectancy of more than 12 wk and a performance status of 0–3 by Southwest Oncology Group criteria (18). There were no eligibility restrictions based on organ function. All patients had to sign an informed consent form in accordance with the Wayne State University Human Investigations Committee.

Intervention

Tablets containing 50 mg of soy isoflavones (Novasoy®) were provided by Archer Daniels Midland Company (Decatur, IL). Each tablet contains 50 mg of total soy isoflavones at a ratio of 1.1:1:0.2 for genistein:daidzein:glycitein; thus, each 50-mg tablet provides roughly 25 mg of genistein. The isoflavones are present in both glucosylated, that is, conjugated (40%), and aglycone, that is, unconjugated (60%), forms. Other contents of the tablets were soya saponins (30–40%), protein (9%), sugars (<1%), fat (<1%), and other minor constituents. Patients had to take two 50-mg Novasoy® tablets twice daily (it could be taken in one dose or two divided doses with meals). The choice for the daily 200-mg dose was based on the desire to achieve a pharmacologic effect in these patients with relatively advanced disease. Because the Japanese diet contains 30–50 mg of daily intake of soy isoflavones, we selected a dose that is severalfold higher because we used the soy isoflavones with a therapeutic intent in this study. In the absence of treatment toxicity or clinical disease progression, patients were required to stay on the study for a minimum of 3 mo to be considered as having had an adequate therapeutic trial. This minimum treatment requirement was arbitrarily chosen to allow adequate time for this dietary compound to act, as it was not expected to result in a rapid response in the disease. Patients were to be treated for a maximum of 6 mo. However, patients responding to treatment beyond the maximum 6-mo study period were allowed to continue Novasoy® at the discretion of the treating physician. Many patients elected to continue the study tablets beyond the 6-mo duration of the study and were followed by the study investigators. Patients in group I (untreated Pca) were treated for only 3 mo if standard therapy was deemed medically indicated by the managing physician.

To verify compliance patients were given a medication calendar and were asked to check the appropriate boxes when they took the study tablets. Patients were advised to make up for a missed pill each day. A pill count on returned bottles was made and compared with the calendar. Patients taking less than 75% of the prescribed dose were counseled to practice stricter compliance. If, on the next monthly visit, there was a similar finding the patient was to be taken off protocol.

Clinical Evaluations and Toxicity Assessment

Potential adverse effects were monitored and reported according to the National Cancer Institute (NCI) toxicity guidelines (19). Because Novasoy® is considered a dietary supplement and the duration of the intervention was short, we did not expect toxicity; therefore, no dose modifications were allowed. In addition to a history and physical examination, baseline assessments included complete blood count with differential count, blood chemistry profile (SMA-12), serum electrolytes, and testosterone levels. These were repeated at the end of the 1st mo and at 3 and 6 mo. Serum PSA levels were measured at baseline and monthly while on study. Blood samples for serum isoflavone levels were obtained monthly for the first 3 mo and at 6 mo. To explore the possible mechanisms of action of isoflavones in patients with Pca we also performed exploratory evaluations of biomarkers with possible association with Pca progression and isoflavone action. Therefore, blood samples for IGF-1, IGFBP-3, and oxidative stress markers were obtained at baseline and at 3 and 6 mo or at the time of removal from study, if earlier. All patients were required to have baseline radiological evaluation, including bone scans and CT scan of the abdomen and pelvis, for disease assessment; these were repeated if clinically indicated.

Study Design and Endpoints

Based on our preclinical data we hypothesized that a daily intake of 200 mg of soy isoflavones would result in decreased or stabilized serum PSA levels, which could also possibly be associated with clinical improvement in some patients. Thus, the primary objective of this pilot study was to assess if soy isoflavone supplementation will decrease serum PSA levels

in patients with Pca. The secondary objectives were assessment of potential toxicity of soy isoflavone intake and the modulation of biomarkers (IGF and oxidative stress markers) by soy isoflavones.

This trial was designed prior to the publication of the "NCI consensus PSA response criteria," therefore, the following PSA response definitions were utilized. Complete PSA response was defined as normalization of PSA (<4 ng/ml, except for patients with history of radical prostatectomy where normalization is ≤0.4 ng/ml) sustained for three successive determinations at a minimum of 2 wk apart. Partial PSA response was defined as ≥50% reduction of PSA sustained for at least three successive determinations (each at a minimum of 2 wk apart). Stable PSA was defined as any PSA change that does not qualify for complete (CR) or partial response (PR) or progressive disease. PSA progression was defined as two PSA values at least 2 wk apart with >50% increase over the minimum PSA level observed during the study.

Serum Isoflavones

Total serum isoflavones were measured using minor modifications of the validated procedure previously published (20). Off-line solid phase extraction of total serum isoflavones was performed following enzymatic deconjugation (Isolute ENV+, 25 mg, Jones Chromatography, Lakewood, CO) followed by quantification of genistein and daidzein using isotope dilution LC-ES/MS with d4-genistein and d3-daidzein internal standards. The method detection limit was approximately 0.02 μM and the inter- and intra-assay precision and accuracy was ±5–10%. Quality control procedures included concurrent analysis of isoflavone-fortified human serum and blank serum.

Serum Testosterone

Total serum testosterone was analyzed in the same sample extracts described for isoflavones, although APCI/MS/MS was used (probe temperature 600°C) with multiple-reaction monitoring of transitions for testosterone and d3-testosterone as an internal standard (*m/z* 289→109 and 292→109, respectively). The method was validated using a woman's serum spiked with 5 ng/ml testosterone (inter- and intra-assay precision of ±15%), and the detection limit was 0.03 ng/ml for 100-μl samples.

Plasma IGF-1 and IGFBP-3 Levels

Plasma IGF-I and IGFBP-3 were assayed by ELISA as previously reported (21).

DNA Oxidation Marker

Enzymatic hydrolysis of DNA combined with GC-MS detection was utilized to quantify 5-hydroxymethyldeoxyuridine as previously reported (22).

Statistical Analysis

Linear mixed-effects modeling was used to test the hypothesis that soy supplementation reduces the rate of PSA rise in patients with Pca. Analysis was stratified by the prior treatment received. PSA measurements within 1 yr prior to intervention were analyzed as baseline levels. Only the PSA measurements within the maximum treatment time of the 6-mo study period were analyzed as postintervention data. Patients in our study had different numbers of repeat PSA levels measured, and all patients did not have their PSA levels measured at precise intervals. Mixed-effects models provide a useful alternative to classical multivariate regression techniques for modeling such data. Our model included random patient-specific intercept and slope to account for the natural heterogeneity in the population. This heterogeneity would be expected due to uncontrolled factors affecting the natural course of Pca in our patient population. The fixed-effects part of our model included time-effect, effect of the soy isoflavone intervention, and Time × Intervention interaction. Logarithmic transformation of the outcome variable was considered to achieve a better model fit. For the covariance model, we fitted several structures, and the model for final inference was selected based on Akaike's Information Criterion (AIC) and Schwarz' Bayesian Criterion (BIC). All analyses were performed using PROC MIXED in SAS. Baseline measures of genistein, daidzein, testosterone, IGF-1, IGFBP-3, and 5-OH-mdU were compared with postintervention values using paired *t*-test.

Results

Patient Characteristics

From January to July of 1999, 41 patients with Pca and rising PSA were enrolled. Patient characteristics are shown in Table 1. The median age was 73 yr, and the median PSA was 13.3 ng/ml. Of the 41 patients, 4 were in group I, 18 were in group II, and 19 were in group III. Among group III patients, 14 had metastatic disease and 5 had nonmetastatic (that is, no radiological findings of metastases) androgen-independent Pca. All group III patients had castrate range testosterone.

Intervention

Soy isoflavone supplementation was given on study with a median duration of 5.5 mo (range = 0.8–6 mo). If subjects had stable disease and wished to continue supplementation beyond the 6 mo required by the protocol, they were allowed to do so. However, for the purpose of the efficacy analysis, PSA data collected on these patients beyond the 6 mo on protocol were not used so as not to bias results. Patients with disease progression by PSA or clinical criteria were taken off the study and placed on other treatments. Overall, 26 patients received between 3 and 6 mo of supplementation, 7 patients

Table 1. Patient Characteristics ($n = 41$)

Median age (yr)	73 (range 55–82)
Race (*n*)	
Caucasian	19
African-American	21
Asian	1
Treatment status (*n*)[a]	
Group I	4
Group II	18
Group III	19
Baseline serum PSA (ng/ml), median (range)	13.3 (0.5–5260)
Group I	43.3 (7.7–106.2)
Group II	4.0 (0.5–150)
Group III	42.4 (3.1–5260)
Median number of days from the start of observation to the start of intervention (range)[b]	
Group I	276 (167–350)
Group II	238 (127–361)
Group III	121 (34–355)
Median number of days from the start to the end of intervention (range)	
Group I	133 (27–177)
Group II	173 (83–182)
Group III	133 (25–183)

a: Group I: previously untreated patients; group II: failed previous local therapy (surgery or radiation); group III: failed previous hormone therapy.
b: Start of observation is the first PSA rise before registration.

received more than 6 mo of supplementation (off study), and 8 patients received <3 mo of supplementation.

Serum Testosterone and Isoflavone Levels

There were no observed changes in serum testosterone due to the intervention (Table 2). The mean postintervention serum levels of total genistein and daidzein increased by approximately five- to sixfold from the preintervention level ($P < 0.001$, Table 2).

Response

Two patients from group III were not assessable for response. Data from 39 patients were analyzed. The median number of preintervention PSA measurements per patient was 4 (range 2–5), and the median number of postintervention measurements per patient was 5 (range 1–6). Overall, there were no complete or partial PSA responses (as defined in **Methods**) in any of the groups. However, stable PSA (as defined in **Methods**) was observed in 3 of 4 (75%) patients in group I, 15 of 18 (83%) patients in group II, and 6 of 17 (35%) patients in group III. Because of the latter observation we then evaluated the rate of rise in PSA. Overall, there was a decrease in the rate of PSA rise, which was 20% prior to intervention and 6% during the isoflavone supplementation ($P = 0.01$; Table 3, Fig. 1). For group II (androgen-dependent, nonmetastatic PSA relapse) the rate of PSA rise decreased from 14% velocity to 6% velocity ($P = 0.21$) and for group III (androgen-independent, that is, hormone-refractory PSA rise) the rate of rise (that is, velocity) decreased from 31 to 9% ($P = 0.05$) following intervention. Separate analysis was not feasible for group I due to the small number of patients.

Toxicity

A total of 190 patient-months of supplementation were given to the patients enrolled in this study. Soy isoflavone supplement was very well tolerated, with no toxicity attributable specifically to Novasoy treatment. No grade 3 or worse adverse events were observed. Although nonspecific grade 1–2 adverse events were reported, their relationship to the study intervention is unclear, and they appeared to be related to preexisting co-morbid conditions. There were no side effects related to soy isoflavone's estrogenic effects, such as gynecomastia or change in hair growth. There were no digestive effects from study tablets.

DNA Oxidation, IGF-1, and IGFBP-3

There was no significant change in the level of 5-OH-mdU after soy isoflavone supplementation compared with the baseline level. There were no statistically significant changes in the plasma levels of IGF-1 and IGFBP-3 due to intervention. There were no significant correlations between changes in PSA levels and IGF-1, IGFBP-3, or IGF-1/IGFBP-3 ratio. Similarly, no relationships were observed between changes in serum PSA levels and changes in 5-OHmdU levels.

Discussion

We have previously reported data suggesting growth inhibitory effects of soy isoflavones in androgen-sensitive and

Table 2. Plasma Levels of Soy Isoflavones and Testosterone Before and After Soy Supplementation

	Preintervention (mean ± SE)[a]	Postintervention (mean ± SE)[a]	*P*
Genistein (μM)	0.11 ± 0.29	0.65 ± 0.63	<0.001
Daidzein (μM)	0.11 ± 0.20	0.51 ± 0.44	<0.001
Testosterone (ng/ml)	1.40 ± 1.36	1.58 ± 1.56	NS[b]

a: SE, standard error.
b: NS, not significant.

Table 3. Percent PSA Rise Before and After Soy Isoflavone Supplementation[a]

Group	*N*	Preintervention (mean ± SE)[b]	Postintervention (mean ± SE)[b]	*P*
II	18	14% ± 3%	6% ± 3%	0.21
III	17	31% ± 5%	9% ± 8%	0.05

a: Percentages refer to the rate of PSA rise per month. These numbers were derived from the estimates of time effect and time × intervention interaction effect obtained from the linear mixed-effect model fit (see **Statistical Analysis).**
b: SE, standard error.

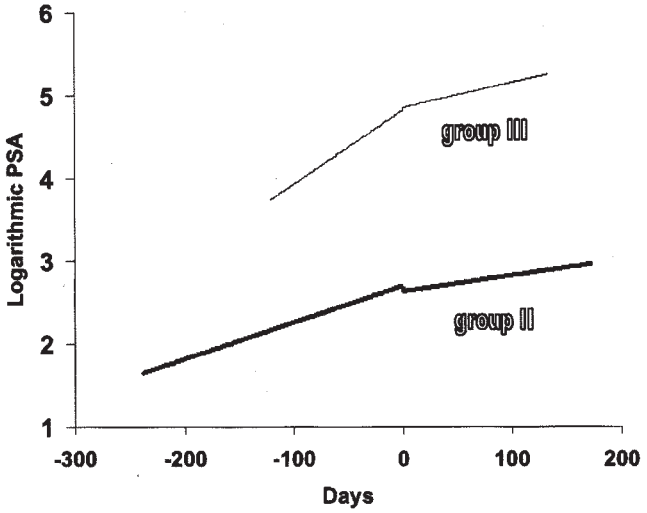

Figure 1. The rate of PSA rise is decreased after soy isoflavone supplementation in patients with androgen-sensitive (group II) and -insensitive (group III) Pca. The 0 on the *x*-axis corresponds to the start of the intervention (soy isoflavone supplementation) point. For each group, the estimated curve extends to the median number of days from the start of the observation to the start of the intervention on the left and the median number of days from the start of the intervention to the end of the intervention on the right (see Table 1 for the group-specific medians).

-insensitive Pca cell lines (15,23). In this pilot clinical trial we sought evidence of clinical activity of soy isoflavone supplementation in patients with Pca. Because soy isoflavones are found in the human diet and have no known significant toxicity, they are attractive compounds in patients with advanced Pca, who may not be candidates for standard therapy because of their old age, indolent disease, and/or co-morbid conditions. Our results confirmed that soy isoflavones are safe, well accepted, and well tolerated by Pca patients. Although no sustained PSA declines qualifying for a PR or CR (see **Methods** for a description of PR and CR) were observed, a significant number of patients had stable PSA levels (83% and 35% of patients in groups II and III, respectively), which suggests some activity of these compounds in this disease. A secondary analysis of the data indicated a reduction in the rate (velocity) of PSA rise, which was seen in groups II and III but was only statistically significant in the latter. This may suggest the possibility of clinical activity; however, this study design precludes definitive conclusions. Therefore, proper clinical phase II trials with more homogeneous patient populations and larger numbers will be required to test this hypothesis. The lack of placebo control is a weakness of this pilot study, and thus randomized placebo-controlled trials should investigate the potential benefit of soy isoflavones in patients with Pca.

Other investigators have also found no decrease in serum PSA levels with soy isoflavone administration (24–26). Urban et al. (24) performed a double-blind crossover pilot study in elderly men with elevated PSA. They administered two soy beverages a day, each containing either 20 g of isolated soy protein, which provided 42 mg of genistein and 27 mg of daidzein daily, or 2.1 and 1.3 mg of the same isoflavones, respectively. After 6 wk of supplementation there was no effect on serum PSA level. Jenkins et al. (25) administered soy foods containing an average of 44 g of soy protein (providing 116 mg of isoflavones) daily for 3 mo to 46 healthy middle-aged men with a range of PSA values and found no significant effect on serum PSA levels. Spentzos et al. (26) conducted a small pilot study with 18 patients who had rising PSA values after radical prostatectomy or curative radiation for localized Pca. They asked the patients first to take a low-fat diet and, upon progression of disease, to proceed to take two packets of soy protein supplement each containing 57 mg of soy isoflavones daily (providing approximately 2:1 ratio of genistein and daidzein) until disease progression. Ten of 17 patients who took the soy isoflavones had a prolongation of the PSA doubling time (P = 0.06). Our results are consistent with these previous reports, which have also found no decrease in serum PSA levels after soy supplementation in patients with Pca and in one study found a decrease in the rate of PSA rise, which was expressed as prolongation of PSA doubling time in that study (26). The study by Urban et al. (24) did not evaluate the rate of PSA rise before and after intervention. Even though there was no decrease in serum PSA in any of these studies, the two studies that assessed the rate of PSA increase before and after intervention both detected a decrease in the rate of PSA rise. The decrease in the slope of PSA rise may indicate a "slowing" in the rate of disease progression because serum PSA has been used as a marker of Pca burden.

The exact mechanism by which soy isoflavones may have resulted in the observed reduction in the rate of rise is not clear. However, soy isoflavones have known biological activities, some of which are mediated by their binding to estrogen and androgen receptors, may in part explain these results. Studies from our institution and others have demonstrated the in vitro inhibitory effect of genistein on PSA synthesis and secretion by Pca cells (23). This was coupled with no alteration in the protein expression levels of another tumor-associated antigen, prostate specific membrane protein (PSM) (23), thus suggesting that the reduction of PSA level is a specific effect of genistein on PSA protein synthesis and secretion. In addition, genistein had in vitro growth-inhibitory and pro-apoptotic effects on hormone-sensitive and hormone-refractory Pca cells (15,23).

In recent epidemiologic studies, relatively high plasma IGF-1 and low IGFBP-3 levels have been independently associated with greater risk of Pca (21). Insulin-like growth factors have mitogenic and anti-apoptotic effects on normal and transformed prostate epithelial cells (27,28); therefore, IGFs may be important in the development and progression of Pca. IGFBPs have opposing actions, in part by binding IGF-1 but also by direct inhibitory effects on target cells. In this study, we also explored possible mechanisms of action of soy isoflavones by measuring serum levels of IGF-1 and IGFBP-3. However, in the present study no significant changes or correlations were observed in serum IGF-1 and IGFBP-3 levels and PSA levels.

Genistein is also a potent antioxidant, inhibiting the oxidation of low-density lipoprotein, alteration in electropho-

retic mobility, and lipid hydroperoxides (9,29–31). Cai and Wei (32) observed significant increases in the activities of antioxidant enzymes catalase, superoxide dismutase, glutathione *S*-transferase, and glutathione peroxidase in various tissues of mice fed genistein in their diet. Studies from our laboratory and others also suggested the antioxidant effect of genistein both in vitro (16,8) and in human volunteers (33). We have also demonstrated that genistein decreases NF-κB DNA binding and abrogates NF-κB activation by oxidative stress–inducing agents H_2O_2 and TNF-α in Pca cells regardless of androgen sensitivity (16). By inhibiting NF-κB activation, soy isoflavones may decrease proliferation of Pca cells and serum PSA level. However, no significant change was observed in oxidative stress marker 5-OHmdU in the current study.

In conclusion, results of this pilot study suggest that soy isoflavone supplementation decreases the rate of rise in serum PSA levels in patients with Pca. This effect is statistically significant in patients with androgen-independent disease. The precise mechanism of this effect is unclear. These results indicate that soy isoflavone supplementation in patients with Pca may slow disease progression and, therefore, may potentially delay the development of symptoms, improve quality of life, and perhaps even prolong survival. Larger clinical trials are warranted to specifically investigate this effect and the potential use of soy isoflavones in the treatment of Pca.

Acknowledgments and Notes

The authors thank Brenda Dickow RN and Shelley Tomalo for their invaluable contributions to study coordination, data abstraction, and management. This article was presented in part at the 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, LA, May 2000 and at the AACR Special Meeting on Prostate Cancer, Naples, FL, December 2001. Supported in part by Cancer Center Grant P30-CA22453–20. Address correspondence to O. Kucuk, Karmanos Cancer Institute, 4100 John R, HWCRC 4th floor, Detroit, MI 48201. Phone: 313–745–2748. FAX: 313–993-0559. E-mail: kucuko@karmanos.org.

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