

Effect of Sulforaphane in Men with Biochemical Recurrence after Radical Prostatectomy

Bernard G. Cipolla¹, Eric Mandron², Jean Marc Lefort³, Yves Coadou⁴, Emmanuel Della Negra⁵, Luc Corbel⁵, Ronan Le Scodan⁶, Abdel Rahmene Azzouzi⁷, and Nicolas Mottet⁸

Abstract

Increases in serum levels of prostate-specific antigen (PSA) occur commonly in prostate cancer after radical prostatectomy and are designated "biochemical recurrence." Because the phytochemical sulforaphane has been studied extensively as an anti-cancer agent, we performed a double-blinded, randomized, placebo-controlled multicenter trial with sulforaphane in 78 patients (mean age, 69 ± 6 years) with increasing PSA levels after radical prostatectomy. Treatment comprised daily oral administration of 60 mg of a stabilized free sulforaphane for 6 months (M0–M6) followed by 2 months without treatment (M6–M8). The study was designed to detect a 0.012 log (ng/mL)/month decrease in the log PSA slope in the sulforaphane group from M0 to M6. The primary endpoint was not reached. For secondary endpoints, median log PSA slopes were consistently lower in sulforaphane-

treated men. Mean changes in PSA levels between M6 and M0 were significantly lower in the sulforaphane group (+0.099 ± 0.341 ng/mL) than in placebo (+0.620 ± 1.417 ng/mL; $P = 0.0433$). PSA doubling time was 86% longer in the sulforaphane than in the placebo group (28.9 and 15.5 months, respectively). PSA increases >20% at M6 were significantly greater in the placebo group (71.8%) than in the sulforaphane group (44.4%); $P = 0.0163$. Compliance and tolerance were very good. Sulforaphane effects were prominent after 3 months of intervention (M3–M6). After treatment, PSA slopes from M6 to M8 remained the same in the 2 arms. Daily administration of free sulforaphane shows promise in managing biochemical recurrences in prostate cancer after radical prostatectomy. *Cancer Prev Res*; 8(8); 712–9. ©2015 AACR.

Introduction

Prostate cancer is the most common solid neoplasm in Europe and the second leading cause of cancer mortality in men (1). In addition to the well-established risk factors, including increasing age, ethnic origin, and heredity, epidemiologic studies suggest that diet and lifestyle are also involved. Obesity, high-saturated fat consumption and a sedentary lifestyle (2) have been shown to increase the risk of prostate cancer. Conversely, regular physical activity (2) and the consumption of fruits and vegetables, particularly those belonging to the cruciferous family, may reduce this risk (3–5). Indeed consumption of cruciferous vegetables has

been reported to reduce the risks of prostate cancer by 40% (3, 4), extraprostatic prostate cancer by up to 40% (6), and progression by 59% (7) in men diagnosed with nonmetastatic disease.

In prostate cancer, primary curative procedures, such as radical prostatectomy and external beam radiation therapy (EBRT), are well-established options for the management of localized disease.

Generally curative, 25% to 40% of men with clinically confined cancer treated with radical prostatectomy will develop biochemical recurrence (8), 34% of which will develop distant metastases within 15 years of total follow-up (9). Pathologic stage, Gleason score, and surgical margin status are independent predictors of biochemical recurrence and are used in nomograms to predict outcome (10). A recent meta-analysis has shown that *GSTP1* hypermethylation may also be a potential predictive biomarker (11).

The management of patients with prostate cancer with recurrence after radical prostatectomy is not clearly defined in nonlocal relapsing patients. A consistently increasing prostate-specific antigen (PSA) level predates overt clinical progression, and a PSA doubling time of less than 6 months is prognostic for metastasis and prostate cancer-specific death (12).

Cruciferous vegetables contain glucosinolates (13), which are a group of sulfur-rich phytochemicals that require conversion to bioactive products, such as isothiocyanates by myrosinase, an endogenous enzyme, released by plant harvesting, processing, or chewing (14). One of the best studied isothiocyanates isolated from cruciferous vegetables is sulforaphane (13), which was originally isolated from broccoli and is produced from glucoraphanin, its glucosinolate precursor (15). Glucoraphanin is

¹Department of Urology, Centre Hospitalier Général de Mont de Marsan, Mont-de-Marsan, France. ²Department of Urology, Clinique du Pré, Technopole Université, Cedex, France. ³Department of Urology, Polyclinique de Lisieux, Lisieux, France. ⁴Department of Urology, Clinique Saint Michel et Sainte Anne, Cedex, France. ⁵Department of Urology, Centre Hospitalier Privé de Saint-Brieuc-Polyclinique du Littoral-Site Sainte Thérèse, Cedex, France. ⁶Department of Oncology and Radiation Therapy, Centre Hospitalier Privé Saint Grégoire, Saint Grégoire, France. ⁷Department of Urology, CHU Angers, Cedex, France. ⁸Department of Urology, CHU Saint Etienne-Hôpital Nord, Cedex, France.

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

Corresponding Author: Bernard G. Cipolla, Centre Hospitalier Général de Mont de Marsan, Av Pierre de Coubertin, Mont de Marsan 40024, France. Phone: 336-600-20015; Fax: 335-580-51760; E-mail: bcipolla@free.fr

doi: 10.1158/1940-6207.CAPR-14-0459

©2015 American Association for Cancer Research.

converted to sulforaphane by myrosinase, but myrosinase is denatured by cooking, and when intact glucoraphanin is ingested, it is eventually converted to sulforaphane by gut microbial thioglucosidases (14, 16).

Sulforaphane can target cancer cells through many demonstrated chemopreventive mechanisms (16). It can inhibit carcinogenic mechanisms such as oxidative stress, phase I enzymes, inflammation angiogenesis, and metastasis and conversely induce "cytoprotective" mechanisms such as phase II detoxifying enzymes, which include glutathione-S-transferases (17), heat shock response, and apoptosis. Among epigenetic DNA alterations observed in human cancers, sulforaphane inhibits DNA methylation (18, 19) and histone modifications.

In patients, *GST isoenzyme P1 (GSTP1)* promoter hypermethylation and therefore gene silencing is present in up to 90% of prostate cancer and is only rarely present in benign prostate tissue (20).

In prostate cancer TRAMP C1 cells, sulforaphane regulates Nrf2's CpGs demethylation and reactivation *in vitro*, suggesting that sulforaphane may exert its chemopreventive effect in part via epigenetic modifications of *Nrf2* gene with subsequent induction of its downstream antioxidative stress pathway (21). *In vivo*, TRAMP mice fed with 240 mg broccoli sprouts/mouse/d exhibited a significant retardation of prostate tumor growth with increased expression levels of Nrf2, HO-1, cleaved caspase-3, cleaved PARP, and Bax proteins (22).

Sulforaphane is also a histone deacetylase (HDAC) inhibitor. HDACs are a group of enzymes that are involved in epigenetic gene silencing (23, 24, 25). For some authors, both DNA demethylation and HDAC inhibition are required to induce complete gene expression of epigenetically silenced genes (26) and sulforaphane fulfills both criteria.

Sulforaphane modulates the androgen receptor either by increasing its degradation through the HDAC6 and heat shock protein pathways (27) or by suppressing androgen receptor transcription (28).

Sulforaphane administration inhibits prostate cancer progression and pulmonary metastasis in TRAMP mice by reducing cell proliferation and augmenting natural killer cell lytic activity (29).

Androgen deprivation is a recommended treatment option; however, its timing after biochemical recurrence remains controversial, particularly for lower risk patients. In a retrospective study comparing early and delayed hormone therapy, early hormone therapy was found to be an independent predictor of delayed clinical metastases only for high-risk patients with a pathological Gleason sum greater than 7 or PSA doubling time of 12 months or less (30).

Watchful waiting may therefore be considered a reasonable option for lower risk patients as early hormone therapy causes hot flashes, loss of libido and sexual activity, osteoporosis, and fatigue with no evidence suggesting prolonged survival.

Therefore, the identification of strategies that delay clinical prostate cancer progression and prolong the interval from treatment failure to hormonal ablation is clearly warranted. Glucosinolates and isothiocyanates could be interesting candidates for such strategies. They have been investigated in clinical trials with no significant reported toxicity (31–33). However, only sulforaphane precursors such as purified glucosinolates or broccoli sprouts and not sulforaphane are currently in intervention trials because sulforaphane is highly unstable in its free form (34). The bioavailability of sulforaphane is therefore

dependent on the presence of myrosinase or gut thioglucosidases, which convert glucosinolate to sulforaphane. In this setting, measurement of urinary levels of dithiocarbamates (sulforaphane metabolites) indicated striking interindividual differences in bioavailability (31).

In an unpublished non–placebo-controlled pilot study of 15 patients with biochemical recurrence following radical prostatectomy, with or without adjuvant or salvage external radiotherapy, treated with 30-mg doses of oral stabilized free sulforaphane daily during 3 months; we observed that sulforaphane was very well tolerated and that the PSA doubling time increased from 10% to 400% for 80% of the patients. Assuming that sulforaphane has a dose- and time-related effect on the reduction of PSA progression, we chose to investigate in this study the effect of a stabilized free-form sulforaphane using a daily dose of 60 mg for a longer treatment time (6 months) in the same population of patients.

Materials and Methods

Patients and recruitment

Ninety patients were enrolled between July 27, 2011 and December 10, 2012 at 14 urological or oncological centers in France. Eligible men presented biochemically recurrent prostate cancer defined by an increasing PSA (at 3 successive measurements) after radical prostatectomy with or without adjuvant or salvage EBRT. Inclusion required the following characteristics: postoperative undetectable PSA, a pT2 or pT3a–b pN0M0 pathologic stage with or without positive surgical margins, a specimen Gleason score ≤ 7 , an increasing PSA ≥ 0.2 and < 5 ng/mL, and a standardized PSA doubling time ≥ 5 and ≤ 36 months, calculated on the Memorial Sloan Kettering Cancer Centre (MSKCC) web site (9, 35). Exclusion criteria were patients with known allergy or intolerance to cruciferous vegetables, severe hepatic, renal or cardiovascular conditions, and those regularly taking other nutritional supplements, such as green tea, turmeric, or pomegranate.

Protocol

The baseline investigations included a medical history, urinary comfort, body weight, body mass index (BMI), physical examination, and blood tests, including blood counts (hemoglobin, red and white blood cells, platelets), hepatic profile, including serum alanine (ALT) and aspartate (AST) transaminases, alkaline phosphatases and total bilirubin, serum sodium, potassium, chloride, and calcium, PSA, and total serum testosterone.

The National Regulatory (ID-RCB: 2011- A00347-34) and Ethics Committees approved the protocol. All of the study participants provided written informed consent.

Randomization, masking and intervention

Eligible patients were randomly assigned to treatment groups by a fax randomization system centralized by the Contract Research Organization (CRO) to ensure that the investigators were blinded to treatment allocation. Participants were randomly assigned to receive either 60 mg of oral sulforaphane (2 tablets containing 10-mg sulforaphane each, 3 times a day) for 6 months, followed by 2 months without treatment. The patients were excluded from the study in cases of significant PSA progression or $>$ grade 2 adverse effects. Treatment was subsequently stopped in both cases and androgen deprivation therapy initiated in case of PSA progression.

Cipolla et al.

Urinary comfort, body weight, and physical examination results were recorded, and blood tests, including PSA and total serum testosterone, were measured at baseline (M0), 1 (M1), 3 (M3), 6 (M6), and 8 (M8) months. To avoid measurement discrepancies, each patient was subjected to blood tests in the same laboratory. To evaluate compliance, the allocated tablet boxes were recovered to count the leftover materials.

Sulforaphane tablet composition

Each tablet contained 10 mg of free stabilized sulforaphane extracted from broccoli seeds (Prostaphane). To improve the stability of sulforaphane, a new cold press process was developed by Nutrinov to produce immediate release tablets of microencapsulated active component powder extract. Placebo tablets were similar in composition and appearance but contained no sulforaphane.

Diet

To avoid any dietary covariant, patients were counseled not to change their usual dietary habits.

Statistical analysis: sample size

The sample size calculation was based on our previous pilot study, which showed that 80 subjects were required to detect a 0.012 log (ng/mL)/month reduction in the slope of log PSA and an estimated 0.017 log (ng/mL)/month SD compared with the placebo at an overall significance level of 0.05 and a power of 85%.

Statistical analyses were performed on the set of all randomized subjects without violation of the inclusion criteria regarding PSA levels (intention-to-treat) and on the subset of intention-to-treat patients for whom the 4 PSA measurements (M0–M1–M3–M6) were available. The patients were considered compliant if they exhibited adequate tablet compliance (>70%). The results are expressed as the means \pm SD and were considered to be statistically significant at *P* levels <0.05. The statistical analyses were performed using JMP-SAS Institute 11.0 for Windows by 2 independent statistical consultancies: SLB Pharma and Effi-Stat.

Primary endpoint

The primary endpoint was the slope of log PSA determined from the values obtained between M0 and M6. As PSA progression is not linear, the log of PSA was chosen for slope calculation and analysis as in the MSKCC algorithm (35). A classical analysis of covariance (ANCOVA), using the initial preintervention slope of log PSA as the covariate, was initially planned. However, the normal quantile plot and the Shapiro–Wilk test for normality both applied on the residuals of the ANCOVA model showed a strong deviation from normality and the presence of 3 influential outliers (1 placebo and 1 sulforaphane higher outlier and 1 sulforaphane lower outlier) at M1. No simple data transformation could solve the normality issue. As a consequence, a nonparametric ANCOVA (Hettmansperger and McKean linear model aligned rank test) was used (36). The Hodges–Lehmann estimator (*d*) and its 95% confidence interval (CI) assessed the treatment effect. To assess sulforaphane activity as a function of time (months), log PSA slopes were calculated by fitting a linear regression of the natural log of PSA measured at M0, M1, M3, M6, and M8. The PSA doubling time was obtained by dividing the natural log of 2 by the slope.

Secondary endpoints

The differences in adverse events between the 2 groups were tested using Fisher and χ^2 tests. PSA progression from baseline at M6 was tested with the Wilcoxon test. The number of men at M6 with arbitrarily defined lower (-10% and below), stabilized ($-10\% < <+20\%$), or increased ($>20\%$) PSA values compared with baseline were analyzed using a χ^2 test.

Results

Patient population

This study accrued and randomized 90 patients between July 27, 2011 and December 10, 2012 at 14 urological centers in France. Eight randomized patients were excluded before start of intervention because of major deviations in eligibility criteria, and one patient withdrew his consent, leaving 81 men receiving by random assignment sulforaphane or placebo. Before unblinding, we excluded 3 patients from the efficacy analysis for violation of the inclusion criteria with respect to PSA levels, but they were retained for the safety analysis. The intention-to-treat population comprised 78 patients (40 placebo and 38 sulforaphane), including 3 patients (1 placebo and 2 sulforaphane) who discontinued study before M6. Thus, for 75 subjects (39 placebo and 36 sulforaphane), the slope of log PSA was calculated by linear regression with use of 4 PSA values from M0 to M6, whereas for 3 subjects, the slopes were calculated by using the 2 or 3 available PSA values (Flow chart; Supplementary Fig. S1).

Two patients with a Gleason score of 8 (both in the sulforaphane arm) were admitted for enrollment as PSA progression was slow and time from salvage EBRT to intervention was long (4 and 6 years). Six patients with post-prostatectomy levels above 0.1 ng/mL were also admitted for the same reasons as above. All but one patient had adjuvant EBRT and time from EBRT to intervention was a median 6 years with a median PSA doubling time of 16 months.

The mean time between radical prostatectomy and the trial was 7 ± 3 years. Ten patients had prior androgen deprivation (in combination with radiation treatment). Mean time from the end of androgen intervention to the trial initiation was 3.7 ± 2 (range, 0.8–7) years. The mean serum total testosterone level at intervention was 4.6 ± 1.4 ng/mL and did not differ statistically from that of the other subjects.

Three patients (1 sulforaphane and 2 placebo) were withdrawn from the study at V1, V3, and V4, respectively, due to significant PSA progression: +60%, +118%, and +240%, respectively, and were commenced on androgen deprivation therapy.

Participants in the sulforaphane or placebo groups were well-matched and did not differ statistically at baseline with respect to demographic, clinical, and pathologic features, blood chemistries, and PSA (Table 1). Although not statistically significant, there were slightly more patients with more serious pathologic features (pT3b, Gleason 4+3, and Gleason 8) in the sulforaphane group. The preintervention slopes of log PSA were 0.063 ± 0.03 log (ng/mL)/month in the placebo group and 0.067 ± 0.04 log (ng/mL)/month in the sulforaphane group, corresponding to PSA doubling times of 14.3 and 14.5 months, respectively.

Primary endpoint: log PSA slopes

The observed median log PSA slopes, *d* treatment effects, and statistical significances are reported in Table 2. The primary

Table 1. Matched baseline clinical, biologic, and pathologic characteristics of the randomly assigned groups

		Placebo (n = 40)	Sulforaphane (n = 38)	P
Age, y	Mean ± SD	70.4 ± 6.8	68.8 ± 6.4	0.291
Weight, kg	Mean ± SD	76.8 ± 9.3	81.7 ± 13.2	0.061
BMI, kg/m ²	Mean ± SD	26.5 ± 2.7	27.8 ± 4.1	0.110
Prostatectomy alone	n (%)	16 (40%)	13 (34%)	0.570
Prostatectomy + EBRT	n (%)	20 (50%)	19 (50%)	1
Prostatectomy + EBRT + hormone therapy	n (%)	4 (10%)	6 (15.8%)	0.444
Age at prostatectomy, y	Mean ± SD	63.2 ± 6.0	61.8 ± 6	0.303
Years between prostatectomy and RTE	Mean ± SD	3.6 ± 2.8	3.6 ± 2.7	0.982
Years between prostatectomy and intervention (M0)	Mean ± SD	7.1 ± 4.4	7 ± 3.5	0.997
Postoperative PSA				0.433
≤0.1 ng/mL	n (%)	36 (90%)	36 (94.7%)	
>0.1 ng/mL	n (%; max)	4 (10%; 0.15)	2 (5.3%; 0.2)	0.642
PSA, ng/mL	Mean ± SD	0.78 ± 0.68	0.74 ± 0.64	
	Median	0.50	0.44	
	Range	0.13–2.77	0.15–2.47	
PSA DT mo	Mean ± SD	14.34 ± 7.54	14.53 ± 8	0.900
	Median	12.14	14.26	
	Range	5.41–33.58	4.17–33.93	
Log PSA slope	Mean ± SD	0.063 ± 0.03	0.067 ± 0.04	0.900
	Median	0.057	0.049	
	[Q1–Q3] [Range]	[0.036–0.087] [0.021–0.128]	[0.036–0.093] [0.020–0.166]	
Testosterone, ng/mL	Mean ± SD	4.45 ± 1.39	4.64 ± 1.55	0.570
	Median	4.49	4.46	
	Range	1.91–7.1	2.1–7.88	
Tumor stage (pTNM)	pT2a	5 (12.5%)	1 (2.6%)	0.288
	pT2b	9 (22.5%)	8 (21.1%)	
	pT2c	14 (35%)	10 (26.3%)	
	pT3a	7 (17.5%)	12 (31.6%)	
	pT3b	5 (12.5%)	7 (18.4%)	
	pN0	36 (90%)	32 (84.2%)	0.445
	pNx	4 (10%)	6 (15.8%)	
	M0	40 (100%)	38 (100%)	1
	SM0	24 (61.5%)	21 (56.8%)	0.535
	SM1	14 (35.9%)	15 (40.5%)	
	SM2	1 (2.6%)	0 (0%)	
	Unknown SM data	0 (0%)	1 (2.7%)	
Gleason scores and (grades)	<6	5 (12.5%)	5 (13.1%)	0.390
	6 (3 + 3)	9 (22.5%)	4 (10.5%)	
	7 (3 + 4)	18 (45%)	17 (44.7%)	
	7 (4 + 3)	8 (20%)	10 (26.3%)	
	8 (4 + 4)	0 (0%)	1 (2.6%)	
	8 (3 + 5)	0 (0%)	1 (2.6%)	

NOTE: Tumor pathological TNM (pTNM) stage.

Abbreviations: DT, doubling time; EBRT, external beam radiotherapy; SM, surgical margins; SM0, no surgical margin; SM1, positive surgical margin ≤ 4 mm; SM2, positive surgical margin > 4 mm.

endpoint of the trial which was to detect a 0.012 log (ng/mL)/month reduction in the slope of log PSA [SD, 0.017 log (ng/mL)/month] compared with that of the group taking placebo, was not reached in the intention-to-treat group (n = 78): P = 0.11, when the slopes were calculated by fitting 4 values of PSA at M0, M1, M3, and M6. In this group, the adjusted median log PSA slope in the sulforaphane group was 38.5% lower than in the placebo group (Fig. 1). The PSA doubling time corresponding to the adjusted median log PSA slope was 28 months for the sulforaphane group and 16.5 months in the placebo group.

A full analysis of the 75 subjects with 4 available PSA measurements at M0, M1, M3, and M6 was performed. In this population, the slope in the sulforaphane group was 37% lower than in the placebo group. The PSA doubling time was 28.9 months for the sulforaphane group and 15.5 months in the placebo group (+86%). Outcomes in this population were close to the intention-to-treat analysis (P = 0.09).

Significantly lower log PSA slopes in sulforaphane group were observed when the slopes were calculated by fitting 3 values of PSA at M0, M3, and M6 and 2 PSA values at M0 and M6. In these

Table 2. Treatment effect assessed by different calculations of log PSA slopes after 6-month intervention

Slope of log PSA (n)	Placebo (n)	Sulforaphane (n)	d (95% CI) in log (ng/mL)/mo	P
M0–M1–M3–M6 (n = 78)	0.0421 (40)	0.0248 (38)	–0.0162 (–0.0374 to +0.0031)	0.11
M0–M1–M3–M6 (n = 75)	0.0447 (39)	0.0240 (36)	–0.0166 (–0.0382 to +0.0022)	0.09
M0–M3–M6 (n = 75)	0.0417 (39)	0.0217 (36)	–0.0180 (–0.0380 to –0.0007)	0.044
M0–M6 (n = 75)	0.0426 (39)	0.0216 (36)	–0.0180 (–0.0381 to –0.0004)	0.04

NOTE: Results for each group are medians of the log PSA slope adjusted to the preintervention slope. The treatment effect (d) and its 95% CI are estimated according to Hodges–Lehmann.

Cipolla et al.

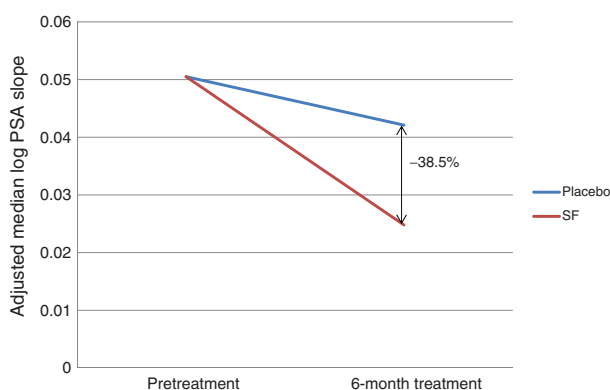


Figure 1. In the 78 ITT population, a 38.5% reduction of the slope of log PSA is observed in the sulforaphane-treated arm compared with the placebo arm, after 6-month treatment.

2 cases, the difference between groups was a decrease of 0.0180 log (ng/mL)/month ($P < 0.05$) and the PSA doubling times were 31.9 and 16.6 months in sulforaphane and placebo groups, respectively (+92%).

Exploratory time sequential analyses

To obtain further insight into sulforaphane effectiveness over time, we examined the PSA slopes in different time subsegments in the full analysis population. Median log PSA slopes were significantly lower in the sulforaphane group than in the placebo group in the segment M3–M6 ($P = 0.011$) and M3–M8 ($P = 0.012$), whereas the segments M0–M1 and M0–M3 were not significantly different between sulforaphane and placebo (Table 3). When treatment was stopped after M6, the M6–M8 mean log PSA slopes were not different between the 2 groups.

Secondary endpoints: PSA progression

The proportion of men with a PSA increase greater than 20% at M6 was significantly greater in the placebo group (71.8%) than in the sulforaphane-treated men (44.4%; $P = 0.0163$; Table 4).

Mean PSA values are reported in Fig. 2 and Table 5. PSA levels increased less in the sulforaphane arm and tended to stabilize after M3. After 6-month intervention, the observed mean PSA change from baseline was significantly lower in the sulforaphane group versus the placebo group: 0.099 ± 0.341 versus 0.620 ± 1.417 ng/mL ($P = 0.0433$); $d = -0.521$ (95% CI = -1.004 to -0.038).

ANOVA revealed that during the preintervention period (from M-14 to M-1), PSA variability did not differ between the 2 groups. After M1, SDs in the PSA levels in the placebo group continued to increase with time, whereas SD stabilized in the sulforaphane group. After M3, the PSA variability was significantly lower in the sulforaphane group than in placebo (Fisher test: $P = 0.0046$ at M3 and $P < 0.001$ at M6 and M8).

The compliance was excellent (96% in each group).

Safety and adverse events

Eighty-one patients were available for adverse event assessment. Thirty-six patients (44.4%) declared at least one adverse event during the trial, 36.5% in the placebo group, and 52.5% in the sulforaphane group. Most adverse events (89%) were grade 1 or at maximum grade 2. There were slightly more gastrointestinal side effects (bloating) in the sulforaphane group (17 vs. 10). One sulforaphane patient withdrew after 1 month due to bowel discomfort. The difference in symptoms between the 2 groups was not statistically significant ($P = 0.149$; χ^2 test). Furthermore, most adverse events were short-term and only reported once during the intervention. Three sulforaphane patients and 1 placebo recipient reported adverse events at 2 or 3 different follow-up assessments. No grade 3/4 adverse events were reported.

There were no statistically significant differences in body weights and BMI, blood counts and chemistries, and testosterone levels between the 2 groups throughout the trial.

Discussion

This trial was designed as a proof of concept study to investigate the effects of a stabilized free form of sulforaphane in a homogeneous population of men with biochemically relapsing prostate cancer after radical prostatectomy, with or without adjuvant or salvage radiation therapy. Although the reduction in log PSA slopes between the sulforaphane (median log PSA slope = 0.0248) and placebo (median log PSA slope = 0.0421) arms was greater (-0.0162) than the expected 0.012 log (ng/mL)/month, the primary endpoint was not reached, most likely due to unexpected PSA variability at M1, to a relatively short intervention time (6 months) and small number of patients involved in the trial. The difference in the log PSA slopes between the 2 arms became significant when PSA values measured at M1 were omitted (M0–M3–M6 or M0–M6) from the calculation. Therefore, there may be possibly an activity signal and further consideration of sulforaphane as a management option for men with biochemical recurrence after definitive therapy is indicated. The resulting PSA doubling time increase was substantial (almost double) and if confirmed in further trials, should be clinically relevant.

The decline in the PSA slopes became prominent after 3 months of sulforaphane treatment suggesting that longer intervention should be considered in future trials. After stopping treatment, the PSA slopes from M6 to M8 were not different between the 2 arms, although there seemed to be a lag effect, as the difference in PSA slopes between M3 and M8 was significant. We initially considered that 6 months of intervention was reasonable as patients on placebo were not exposed to too much risk of prostate cancer progression and that, although no significant toxicity was reported with broccoli extracts or sulforaphane precursors in the literature, no data about long-term free-form sulforaphane intervention are available. Although use of PSA as an endpoint is a recognized limitation, PSA remains the only available follow-up

Table 3. Time sequential analysis of log PSA slopes and treatment effect (d)

Slope of log PSA (n)	Placebo (n)	Sulforaphane (n)	d in log(ng/mL)/mo	P
M0–M3 ($n = 76$)	0.061 (40)	0.046 (36)	–0.015	0.09
M3–M6 ($n = 75$)	0.049 (39)	0.023 (36)	–0.027	0.011
M3–M8 ($n = 72$)	0.049 (37)	0.023 (35)	–0.026	0.012
M6–M8 ($n = 72$)	0.057 (37)	0.032 (35)	–0.025	0.15

NOTE: Results for each group are medians of the log PSA slope adjusted to the preintervention slope.

Table 4. Proportion of men with a higher, stabilized, or lower PSA at M6

PSA M0-M6 progression	Placebo, n (%)	Sulphoraphane, n (%)
Increase (>20%)	28 (71.8%)	16 (44.4%)
Stabilization (>-10% and <20%)	10 (25.6%)	16 (44.4%)
Decrease (<-10%)	1 (2.6%)	4 (11.2%)

NOTE: $P = 0.0163$, χ^2 test.

marker in this setting. Furthermore, PSA doubling time is a validated clinical prognostic marker (12, 30) and increases in PSA levels during prostate cancer patient follow-up is a clinical trigger for management changes.

The compliance was excellent, adverse effects were minimal, and although more men treated with sulforaphane experienced bloating, there were no overall statistically significant differences in symptoms compared with placebo.

The mechanisms of action of sulforaphane in this setting remain to be investigated. Although the serum testosterone levels did not differ between the sulforaphane- and placebo-treated men throughout the trial, we cannot exclude an intracellular modulation of the androgen receptor (27, 28). Induced cell apoptosis (25) could explain short-term (M1) unexpected PSA level variations (declines and increases), as apoptotic cells can release PSA as observed during chemotherapy.

Epigenetic regulation could be the key factor, as *GSTP1* and *GSTM1* hypermethylation and HDACs are prominently involved in prostate carcinogenesis and it has been shown that sulforaphane suppresses both of these mechanisms (24, 25, 37). These questions will be explored in future trials.

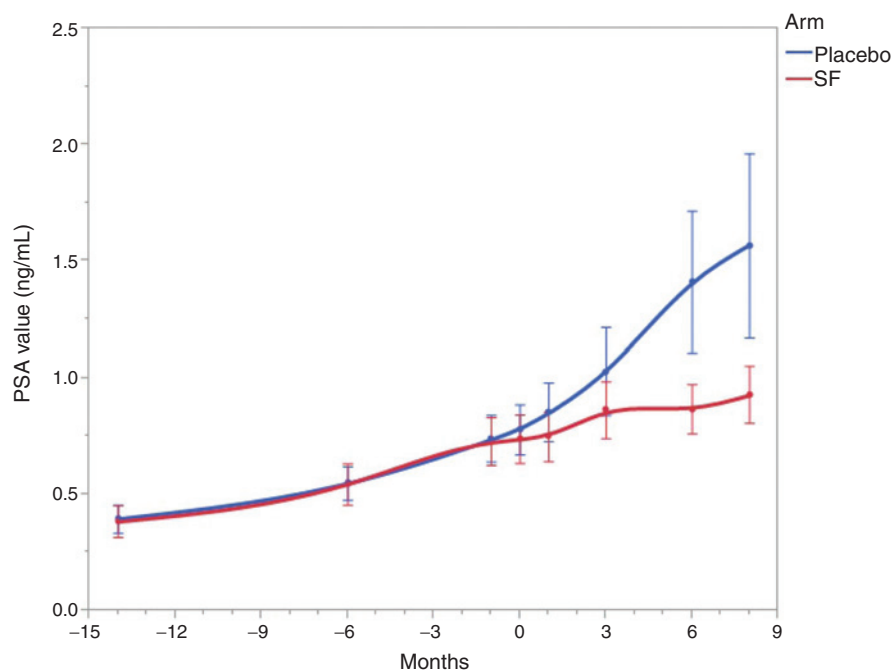
Dietary supplements, herbal remedies, and plant extracts are widely promoted by manufacturers and embraced by patients with cancer (38) who actively seek alternative lifestyle changes and over-the-counter treatments. Experimental data suggest many potential anticancer activities by natural compounds such as genistein and isoflavones from soy (18), polyphenols such as epigallocatechin gallate (EGCG) from green tea (18, 39)

Table 5. Mean \pm SD PSA values before (M-14 to M-1), during (M0-M6), and after (M8) treatment for both randomized groups

Time, mo	Placebo (n)	Sulphoraphane (n)
M-14	0.4 \pm 0.382 (40)	0.38 \pm 0.423 (38)
M-6	0.55 \pm 0.46 (40)	0.54 \pm 0.549 (38)
M-1	0.74 \pm 0.636 (40)	0.73 \pm 0.637 (38)
M0	0.78 \pm 0.683 (40)	0.74 \pm 0.644 (38)
M1	0.86 \pm 0.799 (40)	0.75 \pm 0.661 (38)
M3	1.03 \pm 1.185 (40)	0.86 \pm 0.73 (36)
M6	1.41 \pm 1.897 (39)	0.87 \pm 0.638 (36)
M8	1.57 \pm 2.411 (37)	0.93 \pm 0.726 (35)

NOTE: n, number of patients.

and pomegranate (40), and curcumin from turmeric (41). Nevertheless, clinical evidence is scarce and provides conflicting results for the effects of these compounds. In a double-blind placebo-controlled study, green tea catechins (GTC) significantly prevented prostate cancer development in men with high-grade premalignant prostate intraepithelial neoplasia (42). Two randomized, double-blind, placebo-controlled short-term isoflavone intervention trials before radical prostatectomy have shown that genistein failed to change the PSA levels significantly, even though it modulated the expression of several genes involved in prostate cancer (43, 44). A 6-month double-blind, placebo-controlled intervention study with high-dose soy extracts conducted in 53 men with prostate cancer enrolled in an active surveillance program also failed to show lower PSA levels (45). Conversely, soy extracts appear to be more efficient when combined with other compounds. In a double-blind, placebo controlled, cross-over study, 49 patients with a history of increasing PSA levels after radical prostatectomy ($n = 34$) or radiotherapy ($n = 15$) were allotted to receive a cocktail of soy, isoflavones, silymarin, lycopene, and antioxidants. The study demonstrated a significant decrease in the PSA slope that translated into a 2.6-fold increase in the PSA doubling time (46).

**Figure 2.**

PSA progression (mean \pm SEM) curves in the 2 arms before and during the study.

Only one placebo-controlled study has investigated the effects of broccoli extracts blended with other natural compounds (pomegranate, turmeric, and green tea) in 199 patients with prostate cancer (47). The median increase in the PSA level was 14.7% in the intervention group compared with 78.5% in the placebo group ($P = 0.0008$). Nevertheless, as acknowledged by the authors, the patient population was not homogeneous: the study involved both low-risk patients with prostate cancer managed by active surveillance (59%) but also higher risk patients (41%) on watchful waiting for biochemical recurrence after radical treatment. In that setting, the PSA response in non-prostatectomized patients on active surveillance could therefore be related to the demonstrated anti-inflammatory and antioxidant effects of sulphoraphane, pomegranate, turmeric, and green tea (18).

A stabilized free-form sulphoraphane is a valuable asset in anticancer prevention and therapy. Delivering a predefined dose of sulphoraphane (like any other chemical drug) is mandatory for clinical trials and management. Our future studies will explore its bioavailability in sera of prostate cancer men treated with sulphoraphane. Genomics and proteomics will be performed to have further insight in the mechanisms of action involved. A larger and longer phase III trial is also being designed to confirm its clinical and cost effectiveness.

In conclusion, the effectiveness of a biostable sulphoraphane for decreasing the rate of PSA progression in men with prostate cancer and biochemical recurrence after definitive radical prostatectomy appears promising. The compliance and tolerance were very good. Further studies are required to confirm the clinical importance of this finding.

References

- Boyle P, Ferlay J. Cancer incidence and mortality in Europe 2004. *Ann Oncol* 2005;16:481–8.
- Rebillard A, Lefevre-Orfila L, Guerit J, Cillard J. Prostate cancer and physical activity: adaptive response to oxidative stress. *Free Radic Bio Med* 2013;60:115–24.
- Kolonel LN, Hankin JH, Whittemore AS, Wu AH, Gallagher RP, Wilkens LR, et al. Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. *Cancer Epidemiol Biomarkers Prev* 2000;9:795–804.
- Cohen JH, Kristal AR, Stanford JL. Fruit and vegetable intakes and prostate cancer risk. *J Natl Cancer Inst* 2000;92:61–8.
- Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A prospective study of cruciferous vegetables and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:1403–9.
- Kirsh VA, Peters U, Mayne ST, Subar AF, Chatterjee N, Johnson CC, et al. Prostate, lung, colorectal and ovarian cancer screening trial. Prospective study of fruit and vegetable intake and risk of prostate cancer. *J Natl Cancer Inst* 2007;99:1200–9.
- Richman EL, Carroll PR, Chan JM. Vegetable and fruit intake after diagnosis and risk of prostate cancer progression. *Int J Cancer* 2012;131:201–10.
- Han M, Partin AW, Zahurak M, Piantadosi S, Epstein JI, Walsh PC. Biochemical (prostate specific antigen) recurrence probability following radical prostatectomy for clinically localized prostate cancer. *J Urol* 2003;169:517–23.
- Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *JAMA* 1999;281:1591–7.
- Suardi N, Porter CR, Reuther AM, Walz J, Kodama K, Gibbons RP, et al. A nomogram predicting long-term biochemical recurrence after radical prostatectomy. *Cancer* 2008;112:1254–63.
- Chen R, Ren S, Meng T, Aguilar J, Sun Y. Impact of glutathione-S-transferases (GST) polymorphisms and hypermethylation of relevant genes on risk of prostate cancer biochemical recurrence: a meta-analysis. *PLoS One* 2013;8:e74775.
- Jackson WC, Johnson SB, Li D, Foster C, Foster B, Song Y, et al. A prostate-specific antigen doubling time of <6 months is prognostic for metastasis and prostate cancer-specific death for patients receiving salvage radiation therapy post radical prostatectomy. *Radiat Oncol* 2013;8:170.
- Zhang Y, Talalay P, Cho CG, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci USA* 1992;89:2399–403.
- Fimognari C, Hrelji P. Sulforaphane as a promising molecule for fighting cancer. *Muta Res* 2007;635:90–104.
- Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 1997;94:10367–72.
- Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cell Mol Life Sci* 2007;64:1105–27.
- Talay P, Fahey JW, Holtzclaw WD, Prestera T, Zhang Y. Chemoprotection against cancer by phase 2 enzyme induction. *Toxicol Lett* 1995;82–83:173–9.
- Ho E, Beaver LM, Williams DE, Dashwood RH. Dietary factors and epigenetic regulation for prostate cancer prevention. *Adv Nutr* 2011;2:497–510.
- Wong CP, Hsu A, Buchanan A, Palomera-Sanchez Z, Beaver LM, Houseman EA, et al. Effects of sulforaphane and 3,3'-diindolylmethane on genome-wide promoter methylation in normal prostate epithelial cells and prostate cancer cells. *PLoS One* 2014;9:e86787.
- Phé V, Cussenot O, Rouprêt M. Methylated genes as potential biomarkers in prostate cancer. *BJU Int* 2010;105:1364–70.
- Zhang C, Su ZY, Khor TO, Shu L, Kong AN. Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. *Biochem Pharmacol* 2013;85:1398–404.
- Keum YS, Khor TO, Lin W, Shen G, Kwon KH, Barve A, et al. Pharmacokinetics and pharmacodynamics of broccoli sprouts on the suppression of prostate cancer in transgenic adenocarcinoma of mouse prostate (TRAMP) mice: implication of induction of Nrf2, HO-1 and apoptosis and the

Disclosure of Potential Conflicts of Interest

N. Mottet has received a commercial research grant from and is a consultant/advisory board member of Nutrinov. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: B.G. Cipolla
Development of methodology: B.G. Cipolla, N. Mottet
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.G. Cipolla, E. Mandron, J.M. Lefort, Y. Coadou, E. Della Negra, L. Corbel, A.R. Azzouzi, N. Mottet
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.G. Cipolla, N. Mottet
Writing, review, and/or revision of the manuscript: B.G. Cipolla, E. Mandron, R. Le Scodan, N. Mottet
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.R. Azzouzi

Acknowledgments

Other investigators are Dr. J.M. Leveque (Caen), Dr. M. Allouis (Lorient), Dr. J.P. Graziana (Lorient), Dr. Mhidia (Pontivy), Dr. P. Coloby (Pontoise), and Dr. B. Le Portz (Vannes). The authors thank Anne Laure Serandour of SLB Pharma and JCLemarié of Effi Stat for their statistical analysis and interpretation.

Grant Support

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

Received December 20, 2014; revised March 31, 2015; accepted April 12, 2015; published OnlineFirst May 12, 2015.

- suppression of Akt-dependent kinase pathway. *Pharm Res* 2009;26:2324–31.
23. Myzak MC, Karplus PA, Chung FL, Dashwood RH. A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. *Cancer Res* 2004;64:5767–74.
 24. Myzak MC, Tong P, Dashwood WM, Dashwood RH, Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med* 2007;232:227–34.
 25. Clarke JD, Hsu A, Yu Z, Dashwood RH, Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. *Mol Nutri & Food Res* 2011;55:999–1009.
 26. Hauptstock V, Kuriakose S, Schmidt D, Düster R, Müller SC, von Ruecker A, et al. Glutathione-S-transferase pi 1 (GSTP1) gene silencing in prostate cancer cells is reversed by the histone deacetylase inhibitor depsipeptide. *Biochem Biophys Res Commun* 2011;412:606–11.
 27. Gibbs A, Schwartzman J, Deng V, Alumkal J. Sulforaphane destabilizes the androgen receptor in prostate cancer cells by inactivating histone deacetylase 6. *Proc Natl Acad Sci USA* 2009;106:16663–8.
 28. Kim SH, Singh SV. DL-Sulforaphane causes transcriptional repression of androgen receptor in human prostate cancer cells. *Mol Cancer Ther* 2009;8:1946–54.
 29. Singh SV, Warin R, Xiao D, Powolny AA, Stan SD, Arlotti JA, et al. Sulforaphane inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice in association with increased cytotoxicity of natural killer cells. *Cancer Res* 2009;69:2117–25.
 30. Moul JW, Wu H, Sun L, McLeod DG, Amling C, Donahue T, et al. Early versus delayed hormonal therapy for prostate specific antigen only recurrence of prostate cancer after radical prostatectomy. *J Urol* 2004;171:1141–7.
 31. Kensler TW, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, Ye L, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 2005;14:2605–13.
 32. Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutr Cancer* 2006;55:53–62.
 33. Singh K, Connors SL, Macklin EA, Smith KD, Fahey JW, Talalay P, et al. Sulforaphane treatment of autism spectrum disorder (ASD). *PNAS* 2014;111:1555–5.
 34. Houghton CA, Fasset RG, Coombes JS. Sulforaphane: translational research from laboratory bench to clinic. *Nutr Rev* 2013;71:709–26.
 35. MSKCC. Available from: www.mskcc.org/nomograms/prostate/psa/psa-doubling-time.
 36. Nakonezny PA, Shull RD. MASM26: Hettmansperger and McKean linear model aligned rank test for the single covariate and one-way ANCOVA case (SAS). *J Mod Appl Stat Methods* 2007;6:336–340.
 37. Traka M, Gasper AV, Melchini A, Bacon JR, Needs PW, Frost V, et al. Broccoli consumption interacts with GSTM1 to perturb oncogenic signalling pathways in the prostate. *PLoS ONE* 2008;3:e2568.
 38. Bauer CM, Johnson EK, Beebe-Dimmer JL, Cooney KA. Prevalence and correlates of vitamin and supplement usage amongst men with prostate cancer. *Integr Cancer Ther* 2012;11:83–89.
 39. Suzuki Y, Miyoshi N, Isemura M. Health-promoting effects of green tea. *Proc Jpn Acad Ser B Phys Biol Sci* 2012;88:88–101.
 40. Kroeger N, Belldegrün AS, Pantuck AJ. Pomegranate extracts in the management of men's urologic health: scientific rationale and preclinical and clinical data. *Evid Based Complement Alternat Med* 2013;2013:701434.
 41. Shankar S, Ganapathy S, Chen Q, Srivastava RK. Curcumin sensitizes TRAIL-resistant xenografts: molecular mechanisms of apoptosis, metastasis and angiogenesis. *Mol Cancer* 2008;7:16.
 42. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res* 2006;66:1234–40.
 43. Hamilton-Reeves JM, Banerjee S, Banerjee SK, Holzbeierlein JM, Thrasher JB, Kambhampati S, et al. Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial. *PLoS One* 2013;8:e68331.
 44. Lazarevic B, Hammarström C, Yang J, Ramberg H, Diep LM, Karlsen SJ, et al. The effects of short-term genistein intervention on prostate biomarker expression in patients with localised prostate cancer before radical prostatectomy. *Br J Nutr* 2012;108:2138–47.
 45. deVere White RW, Tsodikov A, Stapp EC, Soares N, Fujii SEH, Hackman RM. Effects of a high dose, aglycone-rich soy extract on prostate-specific antigen and serum isoflavone concentrations in men with localized prostate cancer. *Nutr Cancer* 2010;62:1036–43.
 46. Schröder FH, Roobol MJ, Boevé ER, de Mutsert R, Zuijdgeest-van Leeuwen SD, Kersten I, et al. Randomized, double-blind, placebo-controlled cross-over study in men with prostate cancer and rising PSA: effectiveness of a dietary supplement. *Eur Urol* 2005;48:922–30.
 47. Thomas R, Williams M, Sharma H, Chaudry A, Bellamy P. A double-blind, placebo-controlled randomized trial evaluating the effect of a polyphenol-rich whole food supplement on PSA progression in men with prostate cancer—the UK NCRN Pomi-T study. *Prostate Cancer Prostatic Dis* 2014;17:180–6.

Cancer Prevention Research

Effect of Sulforaphane in Men with Biochemical Recurrence after Radical Prostatectomy

Bernard G. Cipolla, Eric Mandron, Jean Marc Lefort, et al.

Cancer Prev Res 2015;8:712-719. Published OnlineFirst May 12, 2015.

Updated version	Access the most recent version of this article at: doi: 10.1158/1940-6207.CAPR-14-0459
Supplementary Material	Access the most recent supplemental material at: http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/05/13/1940-6207.CAPR-14-0459.DC1

Cited articles	This article cites 46 articles, 10 of which you can access for free at: http://cancerpreventionresearch.aacrjournals.org/content/8/8/712.full#ref-list-1
-----------------------	--

Citing articles	This article has been cited by 6 HighWire-hosted articles. Access the articles at: http://cancerpreventionresearch.aacrjournals.org/content/8/8/712.full#related-urls
------------------------	---

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
----------------------	--

Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org .
-----------------------------------	--

Permissions	To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/8/8/712 . Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.
--------------------	--