Clinical Cancer Research

Muscadine Grape Skin Extract (MPX) in Men with Biochemically Recurrent Prostate Cancer: A Randomized, Multicenter, Placebo-Controlled Clinical Trial

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Abstract

Purpose: MuscadinePlus (MPX), a commercial preparation of pulverized muscadine grape skin, was evaluated as a therapeutic option for men with biochemically recurrent (BCR) prostate cancer wishing to defer androgen deprivation therapy.

Experimental Design: This was a 12-month, multicenter, placebo-controlled, two-dose, double-blinded trial of MPX in 125 men with BCR prostate cancer, powered to detect a PSA doubling time (PSADT) difference of 6 months (low dose) and 12 months (high dose) relative to placebo. Participants were stratified (baseline PSADT, Gleason score) and randomly assigned 1:2:2 to receive placebo, 500 mg MPX (low), or 4,000 mg MPX (high) daily. Correlates included superoxide dismutase-2 (*SOD2*) genotype, lipid peroxidation, and polyphenol pharmacokinetics.

Results: The evaluable population included 112 patients, all treated for at least 6 months and 62% treated for 12 months. No significant difference was found in PSADT change between control and treatment arms ($P = 0.81$): control 0.9 months ($n = 20$; range, 6.7–83.1), low dose 1.5 months ($n = 52$; range, 10.3–87.2), high dose 0.9 months ($n = 40$; range, 27.3–88.1). One high-dose patient experienced objective response. No drug-related CTCAE grade 3–4 adverse events were seen. In a preplanned exploratory analysis, PSADT pre-to-post increase was significant in the 27 (26%) genotyped patients with *SOD2* Alanine/Alanine genotype (rs4880 T>C polymorphism) on MPX (pooled treatment arms; 6.4 months, $P = 0.02$), but not in control (1.8 months, $P = 0.25$).

Conclusions: Compared with placebo, MPX did not significantly prolong PSADT in BCR patients over two different doses. Exploratory analysis revealed a patient population with potential benefit that would require further study. *Clin Cancer Res; 24(2); 306–15. ©2017 AACR.*

Introduction

Men who are experiencing biochemical recurrence (BCR) following definitive local therapy for prostate cancer often seek treatment alternatives that can delay hormonal therapy. They have shown interest in dietary approaches to slow or prevent disease progression without incurring toxicities associated with androgen deprivation therapy (ADT; ref. 1). More than half of those who use dietary supplements started consuming new supplements after being diagnosed with cancer (2). Fifty-one percent of patients with prostate cancer and 39% of patients recently diagnosed with prostate cancer reported using complementary medical products. They used dietary supplements such as herbs, vitamins, and minerals, primarily to boost the immune system and to prevent recurrence (3, 4).

MPX (MuscadinePlus), a commercial preparation of powdered muscadine grape skin, distributed as a dietary supplement, contains ellagic acid and its precursors, the flavonoids quercetin and epicatechin, and the stillbenoid trans-resveratrol, which have demonstrated anticancer activity in prostate cancer cells *in vitro*. Invasive DU145 cells treated with quercetin displayed delayed migration, depressed invasion, and inhibited capillary formation (5). LNCaP cells treated with ellagic acid and resveratrol showed alterations in p53-responsive genes including NFκB p50 and p65 and PPAR families of genes, implying multiple signaling pathways being activated leading to growth inhibition (6). Muscadine grape skin extract antagonized Snail regulation of cathepsin L

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Translational Relevance

Men experiencing biochemical recurrence following definitive local therapy for prostate cancer often employ dietary approaches on the basis of the hypothesis that dietary change may slow or prevent disease progression and thereby delay the initiation of androgen deprivation therapy and its associated toxicities. This randomized, multicenter, placebo-controlled study of 125 patients provides evidence that polyphenol-rich muscadine grape skin extract does not benefit the overall BCR patient population. A preplanned subset analysis using a polymorphism of the superoxide dismutase-2 gene (*SOD2*) as a biomarker shows promising results in the 25% of men with the alanine/alanine genotype of *SOD2*. *SOD2* encodes manganese superoxide dismutase (MnSOD) the primary mitochondrial enzyme that protects cells from oxidative stress. This study illustrates the value of rigorous evaluation of dietary supplements and provides evidence needed to launch a biomarker-driven study of polyphenol-rich foods.

activity, via STAT-3 signaling, leading to decreased bone migration and bone turnover in prostate cancer cells (7) and elicited unfolded protein response–mediated autophagy leading to apoptosis in prostate cancer cells *in vitro* (8).

A phase I trial of MPX in 14 patients experiencing BCR demonstrated safety of up to 4,000 mg/day (eight capsules) as well as adherence at that dose level. Other than grade 1 gastrointestinal symptoms in four patients, patients reported no related adverse events (AEs), and one patient reported improvement of chronic constipation. An exploratory analysis showed a median PSADT lengthening of 5.3 months across all dose levels compared with their baseline (9). Given our earlier findings of the importance of a placebo control in trials of treatments in the BCR patient population (10), we initiated this phase II, randomized dose-finding, placebo-controlled trial of MPX. The primary objective was to define the effects of placebo and two different daily doses of MPX on change in PSADT in men who have rising PSA after initial definitive therapy (radical prostatectomy, radiation, or both ± hormone therapy) for localized prostate cancer. In addition, we explored the association of superoxide dismutase-2 (*SOD2)* genotype and the effect of MPX on PSADT.

Men with the *SOD2* alanine/alanine (Ala/Ala) genotype (rs4880) represent a genetic subgroup that demonstrated a higher risk of aggressive prostate cancer in the presence of low antioxidant status and may benefit more from compounds that reduce oxidative stress (11). Ala/Ala men have greater manganese superoxide dismutase (MnSOD) enzyme activity than Val/Val or Ala/Val men (12), leading to increased DNA damage as measured by 8-hydroxy-2-deoxyguanosine (8-OH-dG; ref. 13). *SOD2* encodes MnSOD, the primary endogenous mitochondrial enzyme. MnSOD converts reactive oxygen species and superoxide anions to hydrogen peroxide and oxygen. The hydrogen peroxide is converted to water via catalase, glutathione peroxidase, or peroxiredoxin. A polymorphism at codon 16 of the *SOD2* gene encodes either alanine or valine. A T-to-C nucleotide polymorphism in exon 2 of the human *SOD2* gene is responsible for a missense mutation that causes a valine (GTC) to alanine (GCC) substitution at amino acid 16 (rs4880, Val16Ala). The allele frequency for alanine is common in the Caucasian population (49%; refs. 14, 15) and African American population (41%–45%; ref. 14), whereas it is 10% to 20% in Asian populations (16, 17).

The *SOD2* Ala/Ala genotype was associated with a >50% reduction in risk of aggressive prostate cancer in the presence of high levels of selenium compared with healthy men with the Ala/Val or Val/Val genotype (11). In the presence of high baseline selenium levels, men with the Ala/Ala genotype showed a reduced risk of presenting with aggressive prostate cancer, while men with Val/Val or Ala/Val genotype showed an increased risk of aggressive prostate cancer (18). In the BCR patient population with the Ala/Ala genotype, the lengthening of PSADT was seen for pomegranate liquid extract, versus placebo (19). We anticipate patients with the Ala/Ala genotype will show greater increase in PSADT in response to MPX treatment than other patients.

Patients and Methods

Patients

The study was a 12-cycle (28 days/cycle), multicenter, placebo-controlled, two-dose, double-blinded trial. Eligible patients had histologically confirmed prostate adenocarcinoma, were ≥18 years of age, had ≥6 months life expectancy, were experiencing BCR defined as a rising PSA on ≥3 time points at least 21 days apart, within 1 year prior to enrollment, and had no radiographic evidence of metastases. Patients were eligible if they had undergone prostatectomy or prostatectomy plus radiation and had baseline PSA >0.4 ng/mL. Patients who had undergone radiotherapy with or without hormonal therapy and had experienced a PSA rise of 2 ng/mL or more above the nadir PSA, per Prostate Cancer Working Group 2 criteria (20), were also eligible. Men were not eligible if they had declining PSA values or if they received therapies that modulate testosterone levels (e.g., androgen ablative/antiandrogen therapy, 5α-reductase inhibitors) within 6 months prior to screening. In addition, men were not eligible if they had prior or concomitant treatment with experimental drugs, high-dose steroids, or any other cancer treatment within 4 weeks prior to the first dose of the study product, or if they had testosterone levels <150 ng/dL, leukocytes <3,000/mcL, platelets <100,000/mcL, or Eastern Cooperative Oncology Group performance status > 2 at screening, or had consumed any muscadine grape–derived products over the past 2 months. In addition, participants were required to continue, while on study, the consumption levels of dietary supplements they had been consuming during the 2 months prior to study initiation and to abstain from commercially available muscadine grape products while on study.

Treatment

Eligible patients identified in physician clinics were randomly assigned 2:2:1 to receive daily doses of eight capsules (4,000 mg daily) of MPX, one capsule (500 mg daily) of MPX plus seven capsules of placebo composed of pulverized rice, or eight capsules of placebo, for up to 12 28-day cycles. To ascertain whether a dose response would be observed, the lowest available dose, 500 mg, was included. It was associated with PSADT prolongation in the phase I trial. The random allocation sequence was defined by the statistician (G.L. Rosner). Patients and physicians were blinded to allocation using opaque capsules in coded bottles allocated by site investigational pharmacy and manufacturer (21). PSA was

measured every 12 weeks until trial completion or progression. Patients remained on study until PSA or radiographic progression, withdrawal for other reasons, or completion of 12 28-day cycles of treatment. In addition, a follow-up visit was scheduled 28 days after completion of treatment.

MPX is a commercial product manufactured by Muscadine Naturals Inc., composed of dried and pulverized skin of *Vitis rotundifolia* (muscadine grapes) of the Noble cultivar. The name MPX was applied to a specific batch of MuscadinePlus, used for experimental purposes in the phase I and phase II trials. The batch designated MPX was manufactured in the regular course of production of MuscadinePlus using standard methods described in the Supplementary Methods. Each 500-mg capsule of MPX contains approximately 1.2 mg of ellagic acid, 9.2 μg of quercetin, and 4.4 μg of trans-resveratrol. The MPX chemical composition and phenolic content are detailed in Supplementary Tables S1.1 and S1.2, respectively. The study drug was provided by Muscadine Naturals Inc.; the study design, data collection, analysis and interpretation were performed by Johns Hopkins researchers. All participants provided written informed consent, and the study was approved by each participating center's institutional review board. The IND (109605) for MPX was held by M.A. Carducci.

Patients' serum PSA and hematologic laboratory assessments were performed and AEs were assessed, using NCI Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) definitions, in clinic at the 1st, 3rd, 6th, 9th, and 12th cycles. Adherence was assessed at each study visit by reviewing daily drug diaries and counting remaining pills. Also at each study visit, changes in concomitant medications and supplements were recorded. At baseline, 3rd and 6th cycle saliva and blood were collected for hormones and correlative studies involving serum lipid peroxidation, pharmacokinetics of polyphenols, and *SOD2* genotyping (baseline only). CT and technetium-99 bone scans were performed at baseline and as clinically indicated.

PSADT

PSADT was defined as the natural logarithm of 2 divided by the slope of the logarithm of serial PSA measures. The slope was estimated via linear regression. If the patient's slope was less than or equal to zero, we assigned them a PSADT of 100 months, which is consistent with the literature (22, 23). Baseline PSADT was calculated using all available PSA measurements in the 12 months prior to enrollment, including screening PSA measurement. The postbaseline PSADT calculation used PSA measurements obtained at baseline (day 0) and on study.

Objective response was defined as a decrease of $\geq$50% in PSA compared with the baseline level. Progressive disease was defined either (i) for subjects who achieved a $\geq$50% decline in PSA, as any increase in PSA value by 50% over the nadir at 6 months on study or beyond, and minimum PSA rise was the greater of 5 ng/dL or back to pretreatment baseline; or (ii) for subjects whose PSA has not decreased by 50%, as an increase in PSA value $\geq$50% of baseline on trial or PSA nadir, whichever is lowest, at 6 months of treatment or beyond. The participant's PSA must also have risen at least 5 ng/dL (confirmed 2 weeks later) or the patient had radiographic or symptomatic documentation of metastatic disease. Stable disease was defined as any response that did not qualify as objective response or progressive disease.

SOD2 genotype

Approximately 5 mL of whole blood at baseline was received from each site within 24 hours of collection and processed immediately for genomic DNA. All samples were lysed with 5× Buffer EL Erythrocyte lysis buffer solution (Qiagen). Samples were further processed according to Qiagen's recommended protocol. DNA purity and concentrations were determined by Nanodrop spectrophotometer at 260 nm and 280 nm. Each DNA sample was stored in Qiagen's EB (50 μL) buffer at −80°C until analysis.

DNA samples were processed by PCR-based allelic discrimination for germline genotyping using TaqMan single nucleotide polymorphism (SNP) genotyping assay from Applied Biosystems. Genotypes were validated using PCR restriction fragment length polymorphism. Detailed methods are presented in Supplementary Methods S2.

Lipid peroxidation

Lipid peroxides are the initial products of lipid peroxidation; their quantification can provide an index of oxidative status. Reduction in lipid peroxides may be correlated with lengthened PSADT (24). We measured both basal and induced oxidative stress levels, with the free radical generator 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), by measuring serum lipid peroxidation in the patient's serum at baseline, 3rd and 6th cycles on study using methods previously described (24, 25).

Ellagic acid, urolithin A, quercetin, and resveratrol pharmacokinetics

We conducted pharmacokinetics studies of the primary MPX polyphenols and their metabolites with the goal of assessing association between serum levels of the polyphenols and change in PSADT, as well as dose effect and adherence. Single blood samples were collected prior to MPX administration at baseline, 3rd and 6th cycles. Concentrations of conjugated and unconjugated ellagic acid, quercetin, resveratrol, and urolithin A in plasma were determined using a validated LC/MS-MS method as described previously (9) and detailed in the Supplementary Methods S3.

Statistical analysis

Participants were stratified by PSADT ($\leq$9 or >9 months) and Grade Groups (Grade Groups 1 and 2, equivalent to Gleason $\leq 3+4$, and Grade Groups 3, 4 and 5, equivalent to Gleason $\geq 4+3$). Gleason 7 (3+4) was separated from Gleason 7 (4+3) to be comparable with the 2013 pomegranate study in the same patient population (26) and because patients with 4+3 cancers have a threefold increase in lethal prostate cancer compared with 3+4 cancers (27). The study started in February 2013 and ended in October 2015, when the last patient completed 12 cycles. The blind was broken in April 2016.

The primary endpoint was the change in PSADT from baseline for each patient. The primary analysis combined patients assigned to the two MPX-containing treatment arms and compared their changes in PSADT to that of the placebo patients. We conducted 2,000 computer simulations under various scenarios to allow us to estimate the design's operating characteristics while also accounting for planned interim analyses and different possible configurations of treatment-related changes in PSADTs. In particular, the study had around 85% power to declare the change in PSADT in MPX-containing

Table 1. Demographics and baseline characteristics

Characteristic	Control ($N = 24$)	500 mg MPX ($N = 56$)	4,000 mg MPX ($N = 49$)	Total ($N = 129$)
Age (years)				
Mean (SD)	69 (7.1)	67 (7.2)	68 (6.9)	68 (7.1)
Median (min, max)	69 (59, 88)	68 (53, 81)	67 (55, 84)	68 (53, 88)
Race				
White	18 (75%)	43 (77%)	38 (78%)	99 (77%)
Black	6 (25%)	12 (21%)	10 (20%)	28 (22%)
Other/unknown	0 (0%)	1 (2%)	1 (2%)	2 (1%)
ECOG				
0	18 (75%)	52 (93%)	39 (80%)	109 (84%)
1	5 (21%)	2 (4%)	8 (16%)	15 (12%)
Gleason score				
$\leq$6, 3+4	11 (46%)	25 (45%)	23 (47%)	59 (46%)
$\geq$8, 4+3	13 (54%)	31 (55%)	26 (53%)	70 (54%)
Baseline PSADT				
$\leq$9 months	13 (54%)	32 (57%)	27 (55%)	72 (56%)
>9 months	10 (42%)	23 (41%)	21 (43%)	54 (42%)
Prior therapy[a]				
Radiation	21 (91%)	49 (89%)	42 (91%)	112 (90%)
Cryotherapy	0 (0%)	2 (4%)	1 (2%)	3 (2%)
Surgery	16 (70%)	40 (71%)	32 (68%)	88 (70%)
Brachytherapy	3 (13%)	5 (9%)	3 (6%)	11 (9%)
ADT	2 (9%)	25 (45%)	19 (40%)	46 (37%)
SOD2 genotype[a]				
Ala/Ala	5 (24%)	12 (27%)	10 (27%)	27 (26%)
Ala/Val	11 (52%)	21 (48%)	22 (59%)	54 (53%)
Val/Val	5 (24%)	11 (25%)	5 (14%)	21 (21%)

[a]Percent calculations for prior therapy and *SOD2* genotype are based on the number of patients in each arm for whom prior therapy and genotypes were recorded.

treatment arms were significantly different from placebo ($n = 20$) in the case where the low-dose arm ($n = 40$) led to an average PSADT increase of 6 months, and the high-dose arm ($n = 40$) had an average PSADT increase of 12 months. The study enrolled 125 men to ensure 100 evaluable patients, allowing for up to 20% dropout. The evaluable population used for the primary analyses included all randomized participants who had at least three on-study PSA measurements, which were separated by at least 10 days, and one of which was $\geq$ 90 days postrandomization. The safety population included all subjects who receive at least one dose of study medication, including those who did not complete the study.

Baseline characteristics were summarized with descriptive statistics. A Wilcoxon rank-sum test was used to compare PSADT change from baseline to postbaseline between the control and pooled MPX arms (two-sided, 0.05 level). The proportion of patients who had a 100% increase of PSADT was compared between the two arms with a χ^2 test. A preplanned subgroup analysis of patients with the *SOD2* Ala/Ala genotype was conducted, assuming a dominant model (28) for rs4880, to assess change in PSADT on treatment. A second subgroup analysis assessed PSADT change in ADT-naïve and ADT-treated patients.

Results

Participant characteristics

Between February 2013 and October 2014, 129 patients were enrolled at six sites and randomly assigned 2:2:1 to receive 4,000 mg MPX, 500 mg MPX, or placebo. Patients were enrolled at Johns Hopkins Sidney Kimmel Comprehensive Cancer Center ($n = 40$, Baltimore and Washington, DC), the Dana-Farber Cancer Center and Beth Israel Deaconess Medical Center ($n = 36$, Boston, MA), Karmanos Cancer Center ($n = 28$, Detroit, MI), Roswell Park Cancer Center ($n = 3$, Buffalo, NY), the Cancer Institute of New Jersey ($n = 21$, New Brunswick, NJ), and Howard University Cancer Center ($n = 1$, Washington, DC).

Baseline characteristics of the evaluable population are shown in Table 1. The median age was 68 (range, 53–88). Of the 129 patients, 28 were African American (22%). Median PSA at baseline was 3.4 ng/mL (range, 0.4–126.8) and median PSADT was 7.5 months (range, 0.9–74.6) at baseline. Median Gleason score was 7 (range, 4–10), and 54% had Gleason scores of 4+3 or $\geq$ 8. Baseline Gleason score, PSADT, and age were similar among the treatment arms. Four patients withdrew consent before being treated, and 13 patients who had fewer than three posttreatment PSA measurements were adjudicated, leaving 112 patients (40 high dose, 52 low dose, and 20 placebo) in the evaluable population (Fig. 1).

Duration of drug exposure

Eighty-seven percent (112/129) of enrolled patients completed six cycles, and 52% (67/129) completed 12 cycles of treatment, with no significant differences in discontinuation rates between the MPX dose groups or between the MPX and control arms. Reasons for discontinuation included (i) disease progression defined as the appearance of radiographically evident metastatic disease and/or physical symptoms felt to be cancer related, or a clinically significant increase in PSA of at least 5 ng/dL, (ii) patient decision to withdraw from the study, (iii) unacceptable AEs, (iv) comorbidities, or (v) other including rising PSA not reaching protocol-defined progression or other changes in the patient's condition. Reasons for discontinuation by treatment arm are summarized in Table 2.

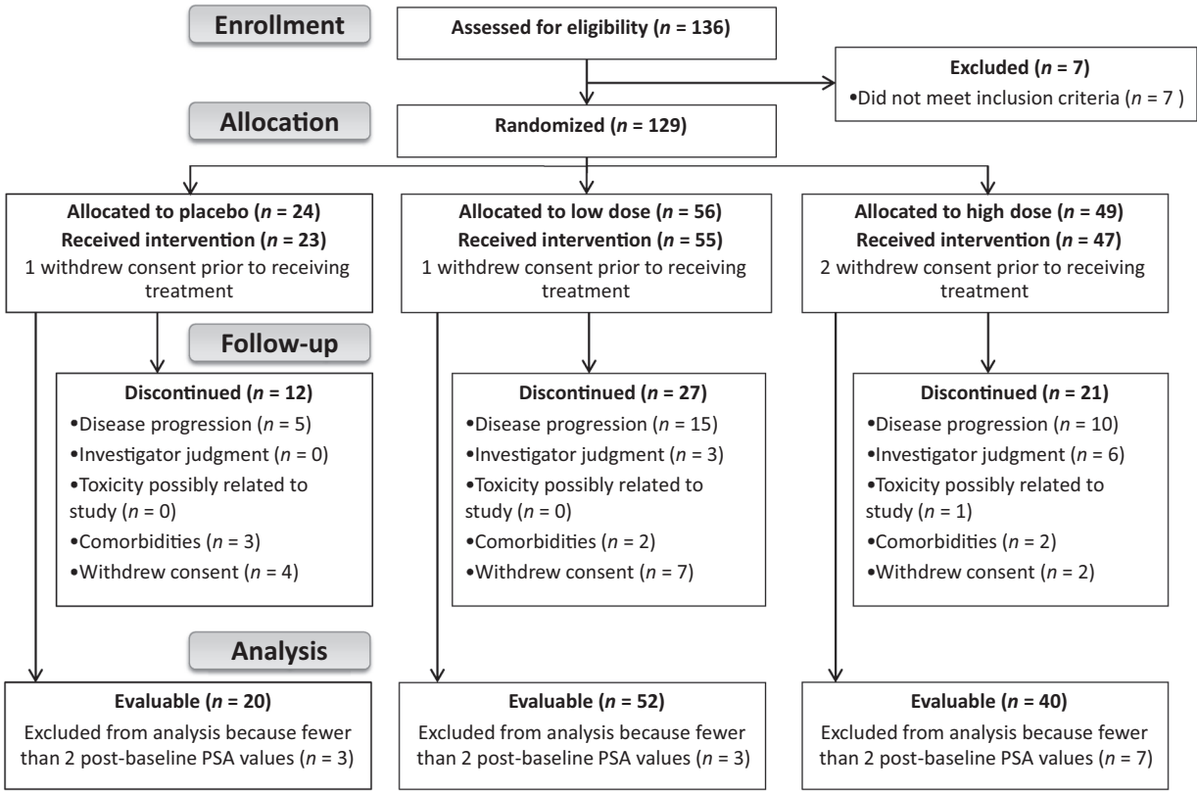

Figure 1.
CONSORT diagram. Patients counted in the follow-up section were evaluable if they completed at least six cycles of treatment prior to discontinuation.

PSA and PSADT outcomes

No significant difference was found in PSADT change between control and treatment arms ($P = 0.81$). For evaluable participants, median PSADT increased by 0.9 months in the control arm ($n = 20$; range, −6.7–83.1), 1.5 months in the low-dose arm ($n = 52$; range,−10.3–87.2), 0.9 months in the high-dose arm ($n = 40$; range, −27.3–88.1), and 0.9 months in the pooled MPX treatment group ($n = 92$, range, −27.3–88.1). The median increase in PSADT was similar in all treatment arms (Fig. 2). Objective PSA decline ($\geq 50\%$) was seen in one patient in the high-dose arm. No objective PSA declines were seen in ether the low-dose or control arms. Declining PSA levels were observed in 7% (8/112) of evaluable patients on study: one in control, five in low-dose, and two in high-dose. Stable disease was seen in 58% of patients (65/112) including 13 patients (65%) in the control arm, 30 patients (58%) in the low-dose arm, and 21 patients (53%) in the high-dose arm. Protocol-defined progressive disease was seen in 46 patients: seven (35%) in the control arm, 22 (42%) in the low-dose arm, and 18 (45%) in the high-dose arm. Post-baseline PSADT of more than 200% of baseline was seen in six of 20 (30%) in the control arm, 12 of 52 (23%) in the low-dose arm, and 10 of 40 (25%) in the high-dose arm.

Table 2. Reasons for discontinuation

Reason	Control	Low dose	High dose	Total
Disease progression	3	7	5	15
Withdrew consent	3	5	4	12
Toxicity	0	0	1	1
Comorbidities	2	2	2	6
Other[a]	3	7	5	15

NOTE: All reasons given for stopping treatment prior to 12 cycles.
[a]Other reasons: rising PSA not meeting protocol defined progression (control: 2, low: 5, high: 2), 4 disenrolled before treatment (control: 1, low: 1, high: 2), patient stopped taking study drug (high: 1), patient transferred to another facility (low: 1)

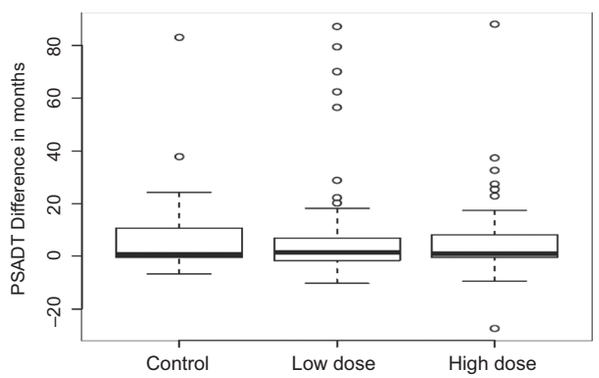

Figure 2.
PSADT change in months from baseline, by treatment arm. This figure includes data for 112 evaluable patients: 20 control, 52 low dose, and 40 high dose.

Table 3. Change from baseline to 6 months in lipid peroxides in serum (μmol/L), by treatment group and *SOD2* genotype

Basal oxidative state						
Month 6 - baseline	**Ala/Ala**		**Ala/Val**		**Val/Val**	
Treatment group	***N***	**Median (range)**	***N***	**Median (range)**	***N***	**Median (range)**
Control	3	3.7 (−14.9–16)	8	4.7 (−2.1–19)	3	7.6 (1.2–39)
Treatment	19	−0.2 (−25.2–22)	38	−1.6 (−20–50.1)	12	−4.9 (−15.4–49.9)
AAPH Oxidative state						
Month 6 - baseline	**Ala/Ala**		**Ala/Val**		**Val/Val**	
Treatment group	***N***	**Median (range)**	***N***	**Median (range)**	***N***	**Median (range)**
Control	3	209.8 (−72.6–217.6)	8	−5.3 (−12.6–20.3)	3	−24.5 (−25.6–160.6)
Treatment	19	11.3 (−266.2–260.5)	38	−3.7 (218.7–273.3)	12	−16.8 (−223.7–107.1)
Change in lipid peroxides in serum (μmol/L)						
Measure/dose level	**Ala/Ala**		**Ala/Val**		**Val/Val**	
		Change in LP		**Change in LP**		**Change in LP**
Basal oxidative state	***N***	**Median (range)**	***N***	**Median (range)**	***N***	**Median (range)**
Control	4	−2.4 (−14.9–16)	8	5.8 (−2.1–19)	3	15.9 (1.2–39)
Low dose	11	1 (−25.2–22)	19	0.7 (−20–50.1)	10	−0.1 (−15.4–39)
High dose	8	9.8 (−3.2–24.6)	19	0.4 (−12.5–14.8)	2	8 (3–13)
AAPH Oxidative state						
Control	4	77.4 (−72.6–217.6)	8	−0.5 (−12.6–20.3)	3	36.8 (−25.6–160.6)
Low-dose	11	18.4 (−202.8–241.3)	19	17.1 (−209.2–262.9)	10	7.8 (−26–160.6)
High-dose	8	37.7 (−266.2–260.5)	19	4.1 (−218.7–273.3)	2	−159.7 (−223.7–95.7)

NOTE: Treatment: combined high- and low-dose MPX, AAPH: 2,2-azobis(2-amidino-propane) dihydrochloride.

SOD2 genotype analysis

Genotyping for the *SOD2* SNP was performed in 102 available samples, of which 27 subjects (26%) had the Ala/Ala genotype. Of the 91 evaluable patients with available samples for *SOD2* genotyping, 23 had the Ala/Ala genotype: four in control, eight in low dose, and 11 in high dose. Hardy–Weinberg equilibrium testing showed no significant departure ($P = 0.63$). PSADT increase for Ala/Ala patients on MPX was not significantly different from PSADT increase for Ala/Ala patients on control [control ($n = 4$) vs. low dose ($n = 8$) $P = 1$, vs. high dose ($n = 11$) $P = 0.94$]. Median pre to post PSADT change in Ala/Ala patients was 6.4 months pre- to post- in the pooled MPX treatment arm ($P = 0.02$) versus 1.8 months in the control arm ($P = 0.25$). High-dose Ala/Ala patients experienced a 10.3-month median increase in PSADT ($P = 0.11$) while low-dose patients experienced a 2.8-month median increase in PSADT ($P = 0.08$). In addition, among the outliers shown above the upper whisker in Fig. 2, Ala/Ala patients were two of five patients with known genotypes in high dose, three of five patients in low dose, and none in control.

ADT-naïve population

Thirty-six percent of evaluable patients on trial had prior androgen deprivation therapy. A subanalysis of the 72 evaluable ADT-naïve patients showed results consistent with the entire population (Supplementary Fig. S4.1). A subanalysis of PSADT change by genotype, in the ADT-naïve population, showed median PSADT change was 1.8 months for control, 5.0 months for low-dose arm for Ala/Ala patients and 15.8 months for high dose, while smaller increases in PSADT in the MPX treatment arms were seen in the Ala/Val and Val/Val genotypes (Supplementary Table S4.1). Among patients who had prior ADT treatment, no Ala/Ala or Ala/Val patients were in the control arm (Supplementary Table S4.2).

Lipid peroxidation

We assayed the *in vitro* serum oxidative stress reduction effect of MPX by evaluating the basal serum oxidative state and the sensitivity to AAPH-induced oxidation of the patients' sera at baseline and six cycles of taking MPX. Compared with baseline, patients' sera showed a decline in basal oxidative state in the combined treatment arm, but not in the control arm, in all three *SOD2* genotypes. No similar declines were seen in the AAPH oxidative state data (Table 3).

Ellagic acid, urolithin A, quercetin, and resveratrol pharmacokinetics

We quantified the plasma concentrations of conjugated and unconjugated resveratrol, quercetin, ellagic acid, and urolithin A in a total of 92 evaluable patients enrolled in the control ($n = 17$), 500 mg ($n = 42$), or 4,000 mg ($n = 33$) dose level. Treatment with MPX resulted in no significant increases in metabolites of MPX. For patients in the control arm, unconjugated and conjugated resveratrol were observed in one patient's sample at baseline. After six cycles, one patient's sample contained conjugated quercetin; the remaining control patients had undetectable concentrations. At the 500-mg dose level, conjugated ellagic acid was observed in one patient after six cycles, while an increase in the number of patients with detectable analyte over time was observed for conjugated quercetin (from 2% at baseline to 10% after six cycles) and conjugated urolithin A (from 33% at baseline to 50% after six cycles). At the 4,000-mg dose level, approximately 10% of patients had detectable unconjugated and conjugated resveratrol at baseline and throughout treatment. The number of patients with detectable conjugated urolithin A increased while on treatment (from 36% at baseline to 76% after six cycles). Despite the increase in the number of patients with detectable conjugated urolithin A while on treatment, there was no significant difference in the exposure observed by dose level on treatment (control: 18.1 ± 29.2 ng/mL baseline vs. 42.1 ± 53.4 ng/mL on treatment; 500 or 4,000-mg: 17.5 ± 24.4 ng/mL baseline vs. 30.5 ± 43.6 ng/mL on treatment).

Safety and tolerability

No deaths or drug-related serious AEs were reported among the patients, and the overall rate of AEs in the control arm was similar

Table 4. Adverse events in intent-to-treat population

	Control	Low dose (500 mg/daily)	High dose (4,000 mg/daily)	Total
Number of patients	23	55	47	125
Patients experiencing adverse events	19 (83%)	38 (69%)	32 (68%)	89 (71%)
Adverse events (definitely related)	0	0	1	1
Adverse events (probably related)	1	2	0	3
Adverse events (possibly related)	3	8	10	21

NOTE: Patients may have experienced more than one adverse event.

to the MPX arms (Table 4). The overwhelming majority of AEs were judged by investigators to be unrelated (69%) or unlikely to be related (22%) to study product in all arms. Most AEs were CTCAE grade 1 or 2. Three patients had grade 1 AEs that were probably drug-related, including diarrhea, flatulence, and fatigue. No patients had grade 2 or higher AEs that were probably drug-related; two patients had possibly drug-related grade 2 dyspepsia and reflux disease. Only one of 270 AEs was definitely drug-related, a grade 1 gastrointestinal AE (flatulence/burping) that occurred in a patient in the high-dose arm causing him to withdraw from the study. Nineteen patients experienced non–drug-related grade 3 to 4 AEs (four in the placebo arm, five in the low-dose arm, 10 in the high-dose arm) including arrhythmia, aortic valve disease, rheumatoid arthritis, congestive heart failure, thrombocytopenia, and myelodysplasia. Two patients experienced unrelated serious AEs (cellulitis and vocal-chord squamous cell carcinoma). No clinical chemistry or hormonal grade 3 to 4 AEs were reported.

Discussion

In this multicenter phase II study, we evaluated the effect of two doses of pulverized muscadine grape skin on PSADT in patients with prostate cancer experiencing BCR. The primary analysis of the clinical trial was negative; the median PSADT increase in patients treated with MPX was nearly identical to the median increase in PSADT in the control arm. Median increases in PSADT from baseline to posttreatment in the pooled MPX arm and in the control arm were statistically significant, but less than 1 month. The small, but statistically significant increase in PSADT in the control arm in this study was consistent with naturally occurring increases seen in other placebo-controlled studies in this patient population (10).

However, in a protocol-defined subset analysis of PSADT change in patients with the *SOD2* Ala/Ala genotype, patients who were treated with MPX experienced a significant 6.4-month median increase in PSADT ($P = 0.02$), while patients on the control arm experienced a 1.8-month median increase in PSADT ($P = 0.25$). The increase in PSADT was greater in the high-dose MPX arm (10.3 months) than in the low-dose arm (2.8 months). Because of small numbers of patients with the Ala/Ala genotype, this difference in the pooled analysis, when compared with placebo, did not reach statistical significance. Findings of increased PSADT in patients with specific biomarkers, based on larger clinical trials, may help those patients and their physicians make informed decisions on whether to supplement their diets with antioxidants. This trial demonstrates safety of MPX, and its findings of larger PSADT increases in patients with the *SOD2* Ala/Ala genotype establish a hypothesis for a placebo-controlled trial of MPX in patients with prostate cancer with the *SOD2* Ala/Ala genotype. A separate trial would be needed to understand the role of *SOD2* genotype in the Asian population both because of low Asian frequency in the U. S. population (no patients identified themselves as Asian in this study) and because of the lower frequency of the alanine allele in Asian populations (16, 17, 29).

An important limitation of this trial was its dependence on PSADT as the primary endpoint. Although PSADT has broad support as a surrogate marker for progression-free survival and overall survival (22, 30, 31), it remains a controversial primary endpoint in clinical trials. Men with a greater PSADT experience a longer metastasis-free survival and overall survival than those with a shorter PSADT (31–33), so prolonging PSADT could be indicative of effective therapy. Furthermore, patients and physicians routinely rely on PSA measurements and PSADT calculations to monitor disease status before progression occurs. Controversy remains, and others have argued for the use of progression-free survival in phase II studies (34), but progression-free survival was not a feasible endpoint for this clinical trial because median bone and soft tissue metastasis-free survival is 10 years in this patient population (32). A related weakness was that prior ADT treatment was allowed, leading to the possibility of greater PSADT shortening and then lengthening during testosterone recovery. However, a subgroup analysis showed that prior ADT treatment was not associated with greater lengthening of PSADT in any of the arms (Supplementary Fig. S4.1).

We hypothesized that ellagic acid, the primary polyphenol in MPX, and its metabolite urolithin A, would increase on treatment. Ellagic acid is metabolized by gut microbiota through a series of enzymatic reactions that remove catechol groups from ellagic acid to generate urolithins (35). We were able to detect conjugated ellagic acid in only one patient, but detected urolithin A in an increasing number of patients between baseline and six cycles on treatment. While it was evident that some patients had residual polyphenols at baseline, likely due to dietary intake of fruits, nuts, and distilled beverages (36), it is promising to note that there was a higher increase in the proportion of patients at both 500 mg or 4,000 mg who had conjugated urolithin A observed in their plasma, and, though a dose effect was observed, it did not reach statistical significance. Polyphenol plasma serum levels were expected to be correlated with genotype and possibly also with adherence. As noted in the results, the ellagic acid, urolithin A, quercetin, and resveratrol concentrations were often undetectable even at the highest MPX dose. We also faced assay constraints (i.e., short long-term freezer stability of analytes and perhaps the assay was not sensitive enough). Therefore, we were unable to utilize drug exposure as an additional measure of MPX adherence and instead needed to rely on the patient reported data, which suggests high compliance. At the dose levels employed in this trial, it is possible that increases in antioxidants are insufficient to induce an effect. However, we would not recommend higher doses because of adherence challenges.

Polyphenolic dietary compounds interact with CYP3A4 and alter its expression and activity (37). However, clinical research evaluating interaction of drugs metabolized by CYP3A4 with pomegranate, a food that shares with MPX ellagitannins as its primary polyphenolic compound, showed that such exclusion based on potential drug interactions was not necessary (38, 39). At the concentrations available after consumption of pomegranate juice, no effect was seen in clearance or plasma levels of midazolam (38) or simvastatin pharmacokinetics (39). Furthermore, interaction with CYP3A4 has not been an issue with other herbal medicines (40). Thus, we did not exclude patients who were taking drugs metabolized by CYP enzymes.

Our primary analysis and subset analyses were consistent with those of the recently reported large trial of pomegranate extract in the same population, which also found no overall effect of the dietary supplement on change in PSADT when compared with placebo (19). Consistency in negative results for the primary analysis of this trial and the other placebo-controlled trial, both with more than 100 patients, suggests that future trials of antioxidant dietary supplements in men with BCR be biomarker driven, using for example, eligibility defined by baseline antioxidant status and/or SNPs that affect antioxidant metabolism (11, 18), to identify subsets of patients more likely to benefit from antioxidants.

The *SOD2* rs4880 genotype may be such a biomarker. Our results suggest this *SOD2* genotype may be predictive of response to MPX treatment, a finding consistent with that of a study of pomegranate extract in the same patient population (19). The impact of the *SOD2* genotype may, however, be modified by baseline antioxidant status, as Ala/Ala genotype was shown to be prognostic for risk of aggressive prostate cancer across quartiles or quintiles of baseline antioxidant status (11, 18). Thus, baseline antioxidant status is a potentially important covariate that should be measured in future studies of antioxidant treatment in men with BCR.

Throughout this article, we have focused on one SNP (rs4880) in the *SOD2* gene. Other SNPs exist in *SOD2*, and in other genes associated with antioxidant pathways. We focused on the rs4880 SNP because it is the most commonly studied SNP in that gene (41), because it was associated with prostate cancer risk in multiple studies (11, 18), and because the *SOD2* rs4880 genotype was associated with increased lengthening in PSADT in men treated with pomegranate in the BCR patient population addressed in our study (19). Furthermore, a study of 16 SNPs in four genes, chosen because of previous reports of their association with prostate cancer risk (*SOD2*) or because those genes were associated with reduced hydrogen peroxide (CAT, GPX1, and GPX4), found only one SNP (in GPX4) significantly modified the relationship between antioxidant status risk of distant organ metastases or death due to prostate cancer. That SNP had never before been shown to be associated with prostate cancer risk, and the authors suggested that, due to the number of SNPs tested, the relationship may be one of chance and should be interpreted cautiously (42).

Recently, a prospective study of pomegranate extract in 183 men with rising PSA following definitive therapy for prostate cancer reported that patients with the *SOD2* Ala/Ala genotype treated with the extract experienced a 12-month increase in median PSADT from 13.6 at baseline to 25.6 months ($P = 0.03$), while Ala/Ala men on placebo experienced a 1.8-month increase in median PSADT from 10.9 months at baseline to 12.7 months ($P = 0.22$; ref. 19). Thus, sufficient hypothesis-generating data now exist to justify a large trial assessing the impact on PSADT of polyphenol-rich food supplements in men with the *SOD2* Ala/Ala genotype who are experiencing rising PSA following local therapy. Such a study should include a correlative analysis of baseline antioxidant status to determine whether the effect of polyphenol-rich food supplement treatment is modulated by antioxidant levels. However, for an unselected patient population, MPX is not recommended.

Disclosure of Potential Conflicts of Interest

W.D. Wagner holds ownership interest (including patents) in Muscadine Naturals Inc. G.L. Rosner is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other authors.

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