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ORIGINAL ARTICLE

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A phase II randomized placebo-controlled trial of pomegranate fruit extract in men with localized prostate cancer undergoing active surveillance

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Abstract

Introduction or Objective: Men with favorable-risk prostate cancer (PCa) on active surveillance may benefit from intervention strategies to slow or prevent disease progression and the need for definitive treatment. Pomegranate and its extracts have shown antiproliferative and proapoptotic effects in cell lines and animal models, but its effect on human prostate cancer as a target tissue remain unclear. Objectives of this trial include pomegranate's ability to alter serum and prostate tissue biomarkers and the ability of an active surveillance cohort to adhere to a chemoprevention trial for 1 year.

Methods: Men with organ-confined, favorable-risk PCa on AS were randomly assigned to receive pomegranate fruit extract (PFE) 1000 mg ($n = 15$) or placebo ($n = 15$) once daily for twelve months. Prostate biopsies were performed at study entry and upon completion of the 1-year intervention. Plasma and urinary biomarkers were analyzed utilizing immunoassays and HPLC. Tissue proteins were assessed by immunohistochemistry (IHC) and measured by automated quantitation.

David Jarrard and Mikolaj Filon contributed equally to this work.

Results: PFE was well-tolerated with no significant toxicities. One patient withdrew before study initiation and 29 completed the 1-year intervention. No differences in plasma insulin-like growth factor-1 (IGF-1) levels, prostate-specific antigen doubling time, or biopsy kinetics were observed. Metabolites including urolithin A and urolithin A-gluc were detected more frequently in the PFE arm in both urine and plasma ($p < .001$ and $p = .006$, respectively). IHC analyses revealed reductions from baseline in 8-OHdG (a DNA damage marker) ($p = .01$) and androgen receptor expression ($p = .04$) in prostate tumor associated with PFE treatment.

Conclusion: PFE administration for 12-month was well-tolerated and the protocol followed in an active surveillance population. Analyses suggest that PFE contains bioactive compounds capable of altering biomarkers involving oxidative stress and androgen signaling in prostate tumor and normal-appearing adjacent tissue. No alterations in the IGF axis were noted. This finding of study adherence and target activity provides a rationale for the further investigation of PFE in the active surveillance population.

KEYWORDS
active surveillance, pomegranate, prostate cancer

1 | INTRODUCTION

Each year, approximately 175,000 men will be diagnosed with prostate cancer (PCa),[1] yet a significant proportion of these patients will be diagnosed with localized low-grade disease that exhibits an indolent progression. This recognition has had major ramifications on the landscape of PCa management, reflected by an increasing trend of low-risk patients assigned to surveillance protocols. In the analysis of the Cancer of the Prostate Strategic Urologic Research Endeavor Registry in patients stratified as low-risk, the use of surveillance has increased from 6.2% to 40.4% between 2000 and 2013, respectively.[2] This increase was even greater in men aged 75 years or older, with 76.2% managed via active surveillance (AS) or watchful waiting in 2013.[2] The implications of these conservative management strategies are meaningful with regard to quality of life, as they delay or altogether avoid more invasive treatments and their associated side effects.[3] This predilection among older men and the relatively prolonged period before the development of clinically relevant disease provides a compelling opportunity for low-risk chemoprevention strategies to slow or prevent disease progression.

Pomegranate is a naturally occurring fruit containing a rich supply of polyphenols and flavonoids that are well tolerated, and have anticancer properties.[4,5] When measuring the equivalent antioxidant capacity of preparations of pomegranate juice, the polyphenols in commercial pomegranate juice had three times the antioxidant activity of green tea and red wine.[6] Pomegranate and its extracts have been shown to have antiproliferative and proapoptotic effects in PCa cell lines and tumor xenografts.[7] Pomegranate inhibits nuclear factor kB which is involved in inflammation and linked to PCa development and risk of biochemical relapse following prostatectomy.[8–10] Elevated plasma levels of insulin-like growth factor-1 (IGF-1) are strongly associated with PCa development and preclinical studies demonstrate pomegranate strongly increases the expression of binding proteins that function to negate the protumorigenic action of IGF-1.[11–13] In other preclinical studies, pomegranate fruit extract (PFE) extract supplementation inhibited tumorigenesis, metastasis, and improved overall survival.[14]

In early clinical work in PCa patients, pomegranate supplementation in various formulations has been evaluated for its impact on prostate-specific antigen (PSA) kinetics, with reports of increased doubling times (Table 1).[20] Specific to pomegranate extract capsules (POMx, Pom Wonderful), there have been two studies evaluating the effects on PCa, both reporting the supplement being well tolerated. Paller et al.[15] investigated PSA doubling time (PSADT) in patients following initial therapy for up to 18 months and found a significant overall prolongation in the median PSADT, but did not show a difference between dosage arms and did not include a placebo group. The second trial compared 2 g of PFE to placebo for 4 weeks before prostatectomy, investigating tissue biomarkers. They found a significantly greater accumulation of a pomegranate metabolite, urolithin A, in the tissue of the PFE arm, but were unsuccessful in demonstrating a significant difference in their primary endpoint of an oxidative damage tissue biomarker 8-OHdG.[16] More recently, a trial evaluating pomegranate in combination with three other natural compounds for 6 months found slower increases in PSA in AS patients on the combination.[21] However to date pomegranate supplementation alone and its impact on prostate signaling pathways, especially within the human gland, has yet to be investigated in an AS population.

TABLE 1 Baseline demographic and clinical characteristics of placebo and pomegranate fruit extract (PFE) cohorts

Characteristic	Placebo ($n = 15$)		PFE ($n = 14$)		p Value
Age (years)	63.6	±7.2	63.2	±9.9	.91
BMI (kg/m^2)	29.9	±3.1	29.2	±3.1	.71
ECOG status 0	15	(100.0)	14	(100.0)	
Race					
Black or African American	0	(0.0)	1	(7.1)	
White	15	(100.0)	13	(92.9)	
PSA (ng/ml)	6.19	±4.16	9.42	±8.0	.16
Tumor grade group					
1	15	(100.0)	13	(92.9)	
2	0	(0.0)	1	(7.1)	
Positive cores	1.53	±0.74	2.00	±1.24	.39
Total cores[a]	16.1	±4.1	14.7	±3.0	.32
Maximum core involvement (%)[b]	16.0	±11.8	28.8	±17.9	.05
Biopsy tumor involvement (mm)[c]	2.73	±2.53	2.96	±2.52	.60

Note: Data are expressed as mean ± *SD* or number of patients (%).

Abbreviation: PSA, prostate-specific antigen.

[a]Total number of cores at time of biopsy.

[b]Represents individual highest percentage cancer involvement among positive cores.

[c]Cumulative length of cancer present in positive cores.

The goal of this phase II randomized placebo-controlled trial was to characterize the compound's effects on multiple important plasma and tissue markers over the course of a one-year administration period in PCa patients assigned to an AS protocol. We also compared the ability of patients in this AS population to adhere to a chemoprevention protocol for an extended period of time.

2 | MATERIAL AND METHODS

2.1 | Trial design

This National Cancer Institute multicenter trial utilized the University of Wisconsin Chemoprevention Consortium (NCT02095145) with patients enrolling from The University of Wisconsin-Madison, The University of Minnesota and Urology of San Antonio. Eligibility criteria included: AS patients with a histologic diagnosis of organ-confined, low-grade PC with prostate tissue available for biomarker analysis from a biopsy performed within ≤13 months of randomization. Low grade was defined as a Gleason score ≤3 + 3 (grade Group 1) and PSA <10 ng/ml in those <70 years old, or Gleason score ≤3 + 4 (grade Group 2) and PSA <15 ng/ml in those ≥70 years old. Following confirmation of eligibility, baseline labs were drawn and prostate biopsy tissue was reviewed to determine Gleason score (grade group) and tumor burden. Subsequently, participants were randomly assigned to one of two treatment groups: (1) pomegranate fruit extract, one 1000 mg capsule p.o daily for 52 weeks or (2) placebo, one capsule p.o daily for 52 weeks. Subjects were stratified in a 1:1 ratio by tumor volume (≤2 positive cores and ≤10% of any biopsy core volume is adenocarcinoma versus greater than 2 positive cores or greater than 10% of any biopsy core volume is adenocarcinoma). The capsule contained powdered pomegranate extract (POMx, POM Wonderful) found to be well tolerated in patients receiving the agent in the neoadjuvant setting and following primary therapy.[15,16]

Patients returned at 13, 26, and 39 weeks for interim evaluation of adverse effects, safety labs, total PSA, compliance, as well as collection of plasma and urine for biomarker analysis. At the end of study visit, in addition to measures collected at interim visits participants also underwent prostate biopsy for evaluation of tumor burden and tissue biomarker analysis. All patients underwent standard TRUS biopsy at baseline and 1 year with a minimum of 12 cores and without MRI assistance.

The primary objective of the study was to determine the change (from baseline to end of study) in the plasma levels of IGF-1. Secondary objectives included determining the change in serum levels of PSA, testosterone, IGFBP-3, and urinary levels of PFE constituents or metabolites including ellagic acid (EA), urolithin A, and urolithin B. Other targets of interest included prostate biopsy tumor burden metrics (presence of tumor, extent of tumor, and Gleason score) tissue expression of PSA, IGF-1, IGF-1 receptor, IGFBP-3, androgen receptor, caspase 3 (apoptosis), TUNEL (apoptosis), PCNA, Ki-67 (proliferation), and 8-OHdG (oxidative stress).

2.2 | Preparation of plasma and urine samples

Samples were frozen, stored at −70°C for up to 2 years and thawed once before analysis. Extractions were done as previously reported,[22] with additional changes detailed in supplemental materials.

2.3 | Serum/plasma/urine biomarkers

Serum or plasma levels of IGF-1, IGFBP-3, total PSA, testosterone were assessed at baseline, and at week 13, 26, 39, and 52 visits. Levels were measured by a commercially available sandwich immunoassay (Quantikine human, R<D Systems). EA, Urolithin A, and Urolithin B levels in both plasma and urine were analyzed using a Shimadzu HPLC system (LC-20AD with SPD-20A UV Detector), UV absorbance reading at 305 and 366 nm. The lower limit of quantitation is 50 ng/ml for all three metabolites, with intra- and inter-day variability of less than 15% (internal data). EA, urolithin A, urolithin B, and the internal standard 6,7-dihydroxycoumarin (esculatin) were all purchased from Sigma-Aldrich.

Qualitative analysis of EA, urolithin A, urolithin A-glucuronide, urolithin B, urolithin B-glucuronide, dimethyl ellagic acid (DMEA), and DMEA-glucuronide was performed using a Q-Exactive™ Hybrid

Quadrupole-Orbitrap Mass Spectrometer. MS data was analyzed using Thermo Xcalibur 3.0 with Foundation 3.0.

2.4 | Tissue biomarkers analysis

Formalin-fixed paraffin-embedded tissues were utilized for immunohistochemistry. Slide preparation and antigen retrieval were conducted as previously described.[23] Briefly, the slides were taken through routine deparaffinization and rehydration. Automated Immunohistochemistry performed on the Ventana Discovery XT BioMarker Platform. Four double stains were performed on sections using antibodies to cleaved Caspase 3 (apoptosis, Cell Signaling Technology Inc.); Ki-67 (proliferation, Thermo Fisher Scientific); IGF-1 (Novus Biologicals); 8OHdG (Abcam); IGF-1R beta (Thermo Fisher Scientific); androgen receptor (Cell Marque); IGFBP3 (Abcam); and PSA (Cell Marque). Vectra (Perkin Elmer) was used for image acquisition and analysis. A scanning protocol including spectral library was created based on the areas of interest (epithelium vs. stroma) and staining complexity (dual staining in a single section). The stained slides were then loaded onto the Vectra slide scanner and 8-bit Bright Field 20X images were acquired for analysis. The inForm 2.4.2 software was used to segment tissue subcellular compartments (nucleus vs. cytoplasm) and tissue compartments (epithelium vs. stroma), and to measure biomarker expression. Except Ki-67 was measured as positive rate (Ki-67 index), all other biomarkers were measured as normalized optical density per pixel in the regions of interest.

2.5 | Statistical methods

The primary endpoint of the study was the change in plasma levels of IGF-1 from baseline to end of study (52 weeks). The primary and secondary endpoints were summarized by treatment arm with descriptive statistics, both tabular and graphical, and analyzed using a two-sample t-test with normalizing transformation if necessary or Wilcoxon rank-sum test. Incidence of adverse events by CTCAE grade was compared between the two treatment groups using Wilcoxon rank-sum test. $p < .05$ were considered statistically significant without adjustment for multiple tests.

2.6 | Sample size justification

The sample size for each group was based on comparing the change in IGF-1 levels between the PFE and placebo arms. According to enzyme-linked immunosorbent assay analysis, this primary endpoint can be considered a continuous random variable. The sample size justification was thus based on a two-tailed two-sample *t* test of the difference between the two groups at a significance level of 0.05. To detect an effect size of 1.10, that is, the difference in the mean change between the two groups of 1.10 times standard deviations, with power 0.80, or an effect size of 1.17 with power 0.85, the trial required an effective sample size of 14 per group. This large effect size was chosen to detect a very bioactive agent. Assuming a random dropout of up to 5%, 15 subjects per group were to be enrolled for a total of 30 subjects for this exploratory study.

3 | RESULTS

A total of 30 patients were enrolled between December 2014 and January 2017, with 15 in each of the placebo and PFE arms. Following randomization one patient reported currently taking a form of pomegranate supplement which the patient was unwilling to discontinue and therefore withdrew from the study. All remaining patients were deemed compliant and completed the study, however missed doses occurred in eight (53%) and eight (57%) of the placebo and PFE groups, respectively. There was no significant difference between the number of doses missed between treatment arms at any time-point (Table S1).

Clinicopathologic data is summarized in Table 1. Following stratification by biopsy core (≤ or >2 positive cores and biopsy core involvement (≤ or >10%) subjects were randomly assigned to one of two treatment groups: (1) PFE, 1000 mg p.o. taken once daily or (2) placebo PFE once daily for 52 ± 1 weeks. Adverse effects were recorded in 13 patients in the placebo group and 14 from the PFE group (Table 2). There were four patients in the placebo arm and three patients in the PFE arm with CTCAE Version 4.0 grade 3 adverse events. All side effects were felt to be unrelated to administration of study drug. None of the reported adverse effects resulted in discontinuation of treatment at any time.

TABLE 2 Adverse events

	Placebo (*n* = 15)					PFE (*n* = 14)					
	All (%)		1	2	3	All (%)		1	2	3	*p* Value
Hypertension	8	(53)	1	4	3	10	(71)	1	8	1	0.54
Upper respiratory infection	3	(20)	0	3	0	2	(14)	0	2	0	1.00
Myalgia	3	(20)	3	0	0	1	(7)	1	0	0	0.60
Nausea	1	(7)	0	1	0	3	(21)	2	1	0	0.33
Urinary issues	1	(7)	0	1	0	3	(21)	1	2	0	0.33

Note: Data are number of patients (%). Adverse events shown here have a frequency of 15% or higher in either treatment arm.
Abbreviation: PFE, pomegranate fruit extract.

TABLE 3 Preintervention versus postintervention plasma biomarker comparisons

Characteristic	Placebo (n = 15)		PFE (n = 14)		p Value
	Mean	*SD*	Mean	*SD*	
IGF-1 (ng/ml)[a]					
Baseline	89.8	±28.7	86.5	±23.21	.83
EOS	98.3	±25.7	106	±25.1	.57
Change	8.54	±23.1	19.1	±21.2	.19
IGFBP-3 (ng/ml)					
Baseline	2590	±599	2500	±599	.74
EOS	2590	±750	2360	±557	.31
Change	-0.39	±498	−138	±363	.45
IGF-1/IGFBP-3 Ratio					
Baseline	0.037	±0.01	0.037	±0.01	.98
EOS	0.040	±0.01	0.046	±0.01	.16
Change	0.003	±0.01	0.009	±0.01	.40
PSA (ng/ml)					
Baseline	6.19	±4.16	9.42	±7.98	.16
EOS	6.11	±4.90	8.42	±4.67	.12
Change	-0.08	±1.83	-1.00	±5.89	.81
PSA doubling time (weeks)	80.65	±330	164	±535	1
Free Testosterone (ng/ml)					
Baseline	9.73	±3.03	9.37	±5.23	.56
EOS	9.27	±3.42	14.8	±19.8	.95
Change	-0.47	±2.35	5.46	±15.7	.16

Abbreviations: EOS, end of study; IGF-1, insulin-like growth factor-1; PFE, pomegranate fruit extract; PSA, prostate-specific antigen.

[a]Primary endpoint

Plasma biomarkers and chemistries were performed and are presented in Table 3. Analysis of the primary endpoint, the change in plasma IGF-1 levels from baseline to EOS, did not significantly differ between the two groups ($p = 0.19$). Change in plasma IGFBP-3 ($p = 0.45$) nor the ratio of IGF-1 to IGFBP-3 differed between the groups ($p = 0.40$). No significant changes plasma free testosterone ($p = 0.16$), PSA ($p = 0.81$), or PSA doubling time ($p = 1.00$) was detected.

To assess the ability of PFE to be absorbed, urine and plasma levels of PFE constituents and metabolites were analyzed at all visits (Table S1). Using mass spectroscopy analysis, greater frequencies of detectable metabolites were found in patients receiving PFE at multiple points. These included urine and plasma urolithin A ($p < .001$ and $p = .01$, respectively), urine and plasma urolithin A-glucuronide ($p = .002$ and $p < .001$, respectively), as well as urine DMEAG ($p < .001$) (Table S2). Quantitative analysis of urinary metabolites using HPLC (Supplementary Table 3) revealed that the PFE group not only had more frequently detectable levels of urolithin A ($p = .003$), a breakdown product of EA, but higher levels present at all time-points following the baseline visit (all $p < .01$).

TABLE 4 Preintervention versus postintervention prostate biopsy metrics

Characteristic	Placebo (n = 15)		PFE (n = 14)		p Value
Positive cores					
Baseline	1.53	±0.74	2.00	±1.24	.39
EOS	1.47	±1.60	1.57	±2.62	.77
Change	-0.07	±1.67	−0.43	±2.98	.43
Total cores[a]					
Baseline	16.1	±4.1	14.7	±3.0	.32
EOS	16.4	±3.22	14.5	±2.35	.10
Change	0.33	±3.75	−0.21	±2.46	.68
Maximum core involvement (%)[b]					
Baseline	16.0	±11.8	28.8	±17.9	.05
EOS	16.0	±20.5	14.7	±20.5	1.00
Change	0	±19.2	−14.2	±21.5	.06
Biopsy tumor involvement (mm)[c]					
Baseline	2.73	±2.53	2.96	±2.52	.60
EOS	4.61	±7.82	3.49	±7.87	.59
Change	1.88	±8.36	0.34	±8.09	.23
Tumor grade group					
Baseline					
1	15	(100.0)	13	(92.9)	
2	0	(0.0)	1	(7.1)	
End of study					
1	8	(53.3)	7	(50.0)	
2	1	(6.6)	2	(14.3)	
No cancer on EOS biopsy	6	(40.0)	5	(35.7)	

Note: Data are expressed as mean ± *SD* or number of patients (%).

Abbreviations: EOS, end of study; PFE, pomegranate fruit extract.

[a]Total number of cores at time of biopsy.

[b]Represents individual highest percentage cancer involvement among positive cores.

[c]Cumulative length of cancer present in positive cores.

Prostate biopsies were performed both at baseline and at EOS, allowing for a comparison of multiple metrics including number of positive cores, maximum core involvement, total tumor and grade and their change over the treatment administration period (Table 4). There were no significant differences between treatment arms identified. A reduction in maximum core involvement in the PFE group approached significance ($p = .06$). Of note, 36%–40% of patients had no tumor on repeat biopsy, consistent with previous findings in other AS populations.[24]

Vectra automated quantitative analysis was performed permitting tissue segmentation focusing on the epithelial component and quantitation of the nuclear-cytoplasmic compartments.[23] Immunostaining of biopsy tissue at baseline was compared with EOS included 8OHdG, AR, IGF-1, IGF-1R, IGFBP-3, the apoptosis marker caspase 3, proliferation gene Ki-67, and PSA (Table S4). Not all patient samples could be analyzed due to lack of biopsy tumor material. However, the PFE cohort demonstrated a reduction in cellular 8-OHdG, a marker of oxidative

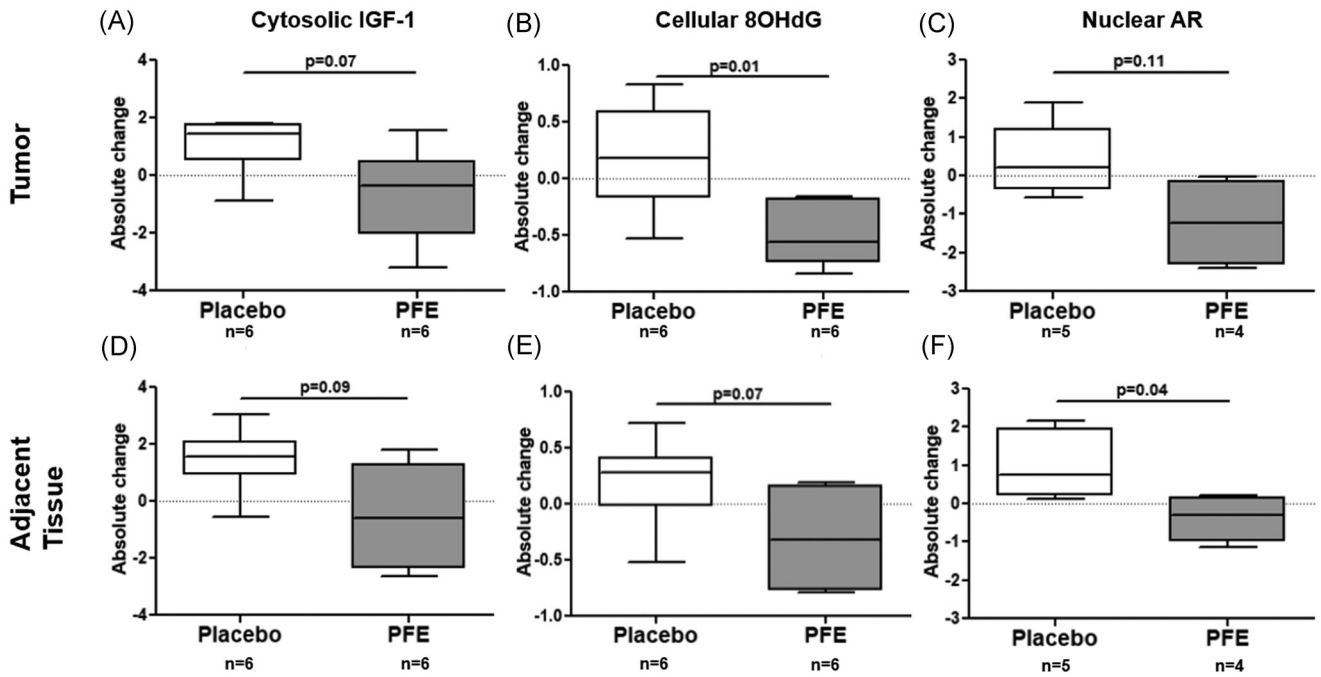

FIGURE 1 Absolute changes in tissue biomarkers from baseline to end of study. Prostate tissues were collected at the time of biopsy and paraffin embedded samples were subjected to immunohistochemistry as described in methods. Inclusion in this comparison required available tumor tissue staining data both at baseline and end of study. Vectra, an automated quantitative system was utilized for analysis.
(A, D) Cytosolic insulin-like growth factor-1 (IGF-1) expression in tumor and benign adjacent tissue (B, E) Cellular 8OHdG, a marker of oxidative stress, in tumor and benign adjacent tissue (C, F) Intra-nuclear androgen receptor expression in tumor and benign adjacent tissue. *p* Values are documented. PFE, pomegranate fruit extract

damage, in tumor tissue ($p = .01$; Figure 1). A reduction in nuclear AR was seen in benign tissue adjacent to tumor in PFE group compared with placebo ($p = .04$), and reduced in tumor ($p = .1$). Although it did not achieve statistical significance there was a trend in the change of Ki-67 from baseline ($p = .07$).

4 | DISCUSSION

In Western populations the initiation of supplements including soy, vitamin D and E, green tea and pomegranate occurs commonly with a new diagnosis of PCa.[25] Pomegranate juice contains anthocyanins, EA derivatives, and primarily hydrolyzable tannins, such as punicalagins which have antioxidant properties.[6] In this consortium chemoprevention study the objective was to determine adherence to taking a supplement over a 1-year trial in AS patients, as well as the systemic absorption and prostate biomarker effects of orally administered PFE. No serious adverse effects attributed to treatment was noted and patient compliance was excellent documented both by patient records and metabolite measurement. Tissue analysis via immunohistochemistry (IHC) showed reductions in several biomarkers including indicators of oxidative stress and androgen signaling. The primary endpoint of producing a significant reduction in the level of plasma IGF-1 compared with the placebo group was not reached. The current trial represents the first randomized trial to evaluate pomegranate supplementation as a sole agent in an AS PCa population.

To date, this trial encompasses the longest continuously documented exposure to pomegranate in an effort to fully evaluate absorption, tolerability, and target penetration. The evaluation of PFE absorption was achieved via analysis of plasma and urine for metabolites of pomegranate, including various urolithins and EA. These metabolites were detected with significantly higher frequency among the PFE arm at multiple time-points (Tables S1–2) and quantifiable urinary levels were also found to be significantly elevated in PFE compared with placebo. IHC biomarker analysis of end-of-study prostate biopsy suggests that PFE administration may reduce tumor 8-OHdG, a marker of oxidative stress, in comparison to placebo (Figure 1). A previous trial evaluating pomegranate supplementation over four weeks before prostatectomy identified a trend towards reduced 8-OHdG in prostate tissue that did not reach significance.[16] That neoadjuvant trial and the current study provide a rationale for the use of 8-OHdG as a primary biomarker endpoint in future studies. In benign tissue there was a reduction in AR expression ($p = .04$) that plays a major role in PCa growth and differentiation. Blocking AR signaling over several years with the 5-alpha reductase inhibitors finasteride or dutasteride demonstrate decreased risk of PC development in several large randomized trials run over 4 years.[26] This study suggests PFE was both successful in being systemically absorbed and enacting physiologic biomarker changes in prostate tissue.

TABLE 5 Summary of findings in pomegranate supplementation as sole agent in prostate cancer

Study/design	N	Population	Dose-intervention	Outcome
Current trial				
Jarrard et al. Phase II double-blind randomized control trial; serum IGF-1	PFE: 14 Placebo: 15	Histologic diagnosis of organ-confined, low-grade PCa, defined as a Gleason score ≤3 + 3 (GG 1) and PSA <10 ng/ml in those <70 years old, or Gleason score ≤3 + 4 (GG 2) and PSA <15 ng/ml in those ≥70 years old on AS.	1 POMx capsule (600 mg GAE) versus placebo daily for 12 months.	No differences in plasma IGF-1 levels or PSADT detected. Urolithin A detected more frequently in the PFE arm in both urine ($p < .001$) and plasma ($p = .006$). Significant reductions from baseline in 8-OHdG (a DNA damage marker) in tumor ($p = .01$) and AR expression in adjacent tissue ($p = .04$) with PFE treatment.
Powdered extract				
Paller et al.[15] Double-blind phase II, dose-exploring trial; PSADT	Low dose: 45 High dose: 47	Histologically confirmed PCa, BCR following radical prostatectomy or external beam radiation therapy, cryotherapy and/or brachytherapy.	1 versus 3 POMx capsules (600 mg GAE each) daily for up to 18 months (58% completed treatment).	Median PSADT lengthened from 11.9 months to 18.5 months after treatment ($p < .001$) with no dose effect ($p = .55$).
Freedland et al.[16] Double-blind randomized neoadjuvant study; oxidative damage tissue biomarker	POMx: 33 Placebo: 36	Histological diagnosis of PCa, scheduled to undergo radical prostatectomy >2 weeks from study entry.	2 POMx capsules (600 mg GAE each) versus placebo daily for at least 2 weeks and up to 4 weeks (mean POMx duration 37 days).	POMx was associated with 16% lower benign tissue 8-OHdG ($p = .095$) and significant increase in detection of Urolithin A, a pomegranate metabolite ($p = .03$), in treatment arm.
Pomegranate juice/liquid extract				
Pantuck et al.[17] Phase II, Simon two-stage single arm clinical trial; PSA progression	46	Diagnosis of PCa, Gleason score ≤7, with rising PSA after surgery or radiotherapy.	8 oz of Pomegranate juice daily (570 mg GAE).	Mean PSA doubling time significantly increased with treatment from a mean of 15 months at baseline to 54 months posttreatment ($p < .001$).
Stenner-Liewen et al.[18] Phase IIb, double-blind randomized study; PSA kinetics	Pomegranate: 45 Placebo: 42	Histologically confirmed PCa, with PSA ≥5 ng/ml, on baseline treatment (including ADT). 68% CRPC	500 ml of juice with added pomegranate (1147 mg GAE) versus juice with placebo daily for 4 weeks; followed by 250 ml of pomegranate juice (573 mg GAE) daily in all subjects for 4 weeks.	PSA progression within the first four weeks was observed in 41% in the control group compared with 38% in the pomegranate group ($p = .83$). No differences were detected between the two groups with regard to PSA kinetics.
Pantuck et al.[19] Double-blind, placebo-controlled study; PSADT	Extract: 102 Juice: 17 Placebo: 64	Histologically confirmed PCa, with rising PSA levels after primary therapy.	8 oz of Pomegranate extract (776 mg GAE) versus placebo daily until PSA progression criteria met. Juice arm discontinued at 1 year.	Median PSADT increased from 11.1 months at baseline to 15.6 months in placebo ($p < .001$) compared with increase from 12.9 months at baseline to 14.5 months in the extract group ($p = .13$) and increase from 12.7 at baseline to 20.3 in the juice group ($p = .004$). However, none of these changes were statistically significant between the three groups ($p > .05$).

Abbreviations: CRPC, castrate resistant prostate cancer; GAE, gallic acid equivalents; IGF-1, insulin-like growth factor-1; PCa, prostate cancer; PSA, prostate-specific antigen; PSADT, PSA doubling time.

Epidemiologically, increased plasma IGF-1 levels, and their concentration relative to IGFBPs, have been linked to a greater risk of developing PCa.[12,27] This family of binding proteins negate the physiologic effects of IGF-1, and themselves regulate cell growth and survival.[28] PFE supplementation results in altered IGF-1 to IGFBP-3 ratio and a inhibition of IGF-I/Akt/mTOR in a mouse model of PCa.[14] These studies provided a rationale for the investigation of IGF-1 as a biomarker of response. We found that the concentrations of plasma IGFBP-3 remained relatively constant over the duration of the pomegranate supplementation. In this cohort PFE did not alter the levels of IGFBP-3, which accounts for 75%–80% of total IGF binding capacity in blood.[29]

Other objectives included assessing the impact of PFE supplementation on PSA kinetics and biopsy metrics. Pomegranate supplementation initially generated attention following reports of substantial lengthening of PSADT in patients with biochemical recurrence following primary treatment.[15,17] (Table 5) However, in a subsequent placebo controlled trial this improvement in PSADT with pomegranate supplementation was not evident.[19] No differences in PSADT between PFE and placebo groups were noted in the current trial (Table 4). Notably, PSA is an imperfect measure of cancer progression. Of note, systemic testosterone levels were also not altered during the course of treatment. Another objective of this study was to evaluate the effect of PFE supplementation on various prostate biopsy metrics including maximum core involvement, total tumor burden, and tumor grade. There were no significant differences in these metrics between the two arms (Table 4). Analysis of prostate biopsies in the current limited cohort does present the issue of sampling error as it has been shown that repeat biopsy fails to detect cancer in roughly 50% of patients.[30]

To properly contextualize these findings as they relate to pomegranate's role in chemoprevention for PCa, it is crucial to consider the limitations of this trial. In the calculation of the sample size for each treatment arm, there was no preliminary data available upon which to estimate treatment effect size. Therefore, to maximize the opportunity of identifying clinically meaningful changes and concurrently ensure prompt trial completion, a large treatment effect size was chosen, reducing the sample size. Additionally, an administration period of 1 year represents a relatively short time to generate large effects, particularly in the context of a disease that has a long natural history. Furthermore, 35.7% and 40% of patients in the treatment arms did not display carcinoma at end of study biopsies; a number that may be improved with modern MRI-guided biopsy techniques. Finally, heterogeneous responses may result to PFE may occur. A variable of interest that was not explored by this study is the effect of manganese superoxide dismutase (MnSOD) status on PSA dynamics in the AS population. It has shown that with pomegranate supplementation following primary therapy for PC, men with the MnSOD Ala/Ala genotype experienced a 12 month increase in PSADT from 13.6 to 25.6 months ($p = .03$) suggesting they represent a subgroup susceptible to low antioxidant status.[19] The MnSOD Ala/Ala genotype has been shown to have greater enzyme activity, conferring increased oxidative toxicity and 8-OHdG formation. Furthermore, PC specific evidence demonstrates that the Ala/Ala group with low antioxidant levels are more likely to develop aggressive PC and allelic status may be prognostic of response to antioxidant therapy.[31,32] This in addition to our evidence calls for further trials to implement evaluating MnSOD genotype status on tissue biomarker alterations with pomegranate in the AS population.

5 | CONCLUSION

This study marks the first randomized, placebo-controlled trial of pomegranate supplementation in an AS population over an extended period. Over the course of 1-year, our data suggest the possibility that this low-risk intervention affects tissue-level changes in markers of oxidative stress and androgen receptor expression. The current trial represents a brief period in terms of the long natural history of PCa and administration of a chemopreventive agent. Given the current biomarker findings it would be of interest to study a longer-term exposure to PFE and determine its capacity to reduce the number of patients transitioning from AS to treatment.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

DATA AVAILABILITY STATEMENT

https://clinicaltrials.gov/ct2/show/results/NCT02095145

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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