Research Paper

Tissue Effects in a Randomized Controlled Trial of Short-term Finasteride in Early Prostate Cancer☆

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ABSTRACT

Background: In the Prostate Cancer Prevention Trial, finasteride selectively suppressed low-grade prostate cancer and significantly reduced the incidence of prostate cancer in men treated with finasteride compared with placebo. However, an apparent increase in high-grade disease was also observed among men randomized to finasteride. We aimed to determine why and hypothesized that there is a grade-dependent response to finasteride.

Methods: From 2007 to 2012, we randomized dynamically by intranet-accessible software 183 men with localized prostate cancer to receive 5 mg finasteride or placebo daily in a double-blind study during the 4–6 weeks preceding prostatectomy. As the primary end point, the expression of a predefined molecular signature (ERβ, UBE2C, SRD5A2, and VEGF) differentiating high- and low-grade tumors in Gleason grade (GG) 3 areas of finasteride-exposed tumors from those in GG3 areas of placebo-exposed tumors, adjusted for Gleason score (GS) at prostatectomy, was compared. We also determined androgen receptor (AR) levels, Ki-67, and cleaved caspase 3 to evaluate the effects of finasteride on the expression of its downstream target, cell proliferation, and apoptosis, respectively. The expression of these markers was also compared across grades between and within treatment groups. Logistic regression was used to assess the expression of markers.

Findings: We found that the predetermined molecular signature did not distinguish GG3 from GG4 areas in the placebo group. However, AR expression was significantly lower in the GG4 areas of the finasteride group than in those of the placebo group. Within the finasteride group, AR expression was also lower in GG4 than in GG3 areas, but not significantly. Expression of cleaved caspase 3 was significantly increased in both GG3 and GG4 areas in the finasteride group compared to the placebo group, although it was lower in GG4 than in GG3 areas in both groups.

Interpretation: We showed that finasteride's effect on apoptosis and AR expression is tumor grade dependent after short-term intervention. This may explain finasteride's selective suppression of low-grade tumors observed in the PCPT.

1. Introduction

Progress in understanding the biology of advanced prostate cancer prompted development of therapy for castration-resistant disease; however, no parallel advances have brought improvement to prevention or treatment of early prostate cancer. This limitation, reflected in the difficulty in interpreting findings of the Prostate Cancer Prevention Trial (PCPT) (Thompson et al., 2003) and the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial (Andriole et al., 2010), led to denial of approval of 5α-reductase inhibitors as prostate cancer preventatives, despite their striking reduction of low-grade cancers. In both studies, the 5α-reductase inhibitors reduced the frequency of low-grade cancers but not potentially lethal high-grade cancers, pointing to the urgent need to elucidate the biologic significance.

Androgen signaling is central to prostate cancer development and progression. A milestone in progression of advanced prostate cancer when it transitions from endocrine-driven to paracrine- or intracrine-driven androgen signaling, with progressive complexities in steroid hormone biosynthesis and alterations of the androgen receptor (AR) (Logothetis et al., 2013). The PCPT, the first study to demonstrate that prostate cancer could be prevented or greatly delayed (Thompson et al., 2003), showed that men taking the type 2 5α-reductase steroid inhibitor finasteride had a relative reduction of 24.8% (P < 0.001) in the 7-year period prevalence of prostate cancer compared with men taking the placebo, a reduction that increased to 30% on assessment of all men who were randomized (Thompson et al., 2013). Paradoxically, incidence of high-grade disease also significantly increased among men on finasteride. Although detection bias appeared, in part, to account for the increase in high-grade disease (Lucia et al., 2007), true induction of de novo high-grade disease could not be ruled out and, therefore, the drug was not granted FDA approval for prostate cancer risk reduction.

The controversy notwithstanding, the PCPT's relevance was made clear in the Reduction by Dutasteride of Clinical Progression Events in Expectant Management (REDEEM) trial (Fleshner et al., 2012). In that study, 302 men with low-grade prostate cancer undergoing active surveillance received three years of treatment with dutasteride or placebo. Dutasteride was associated with a 38% decrease in the cancer detection rate on repeat biopsy at year 3 (Fleshner et al., 2012), supporting the hypothesis that, based on response to a 5α-reductase steroid inhibitor, localized prostate cancer could be dichotomized as either dependent on dihydrotestosterone (DHT) or as able to adapt to DHT depletion.

To improve biologic understanding of the grade effects of finasteride, we undertook a randomized controlled trial of short-term finasteride exposure in men with clinically organ-confined prostate cancer who were scheduled for prostatectomy. We hypothesized that, following a short course of finasteride and preceding detectable morphologic changes, molecular changes associated with high-grade disease would be apparent.

2. Materials and Methods

2.1. Study Design

We performed a randomized, double-blind, placebo-controlled parallel trial comparing the tissue effects of 5-mg finasteride with those of matching placebo given orally daily 4–6 weeks before prostatectomy in patients with clinically organ-confined prostate cancer allocated 1:1 to each group at four academic medical centers. This study is a window-of-opportunity trial, which takes advantage of the interval between a clinic visit and admission to the hospital for prostatectomy, for short-term exposure to the study drug. Approved by the institutional review boards of participating sites and the National Cancer Institute Division of Cancer Prevention (DCP) Protocol and Safety Review Committee, the study was led by The University of Texas MD Anderson Cancer Center Phase I/Phase II Chemoprevention Trials Consortium in Houston. After a review of the purpose, risks, and benefits of the study, all participants signed and received a copy of a written consent. The lead organization's institutional review board provided oversight. Other participating academic centers were Cleveland Clinic in Cleveland, Ohio, The University of Texas Southwestern Medical School in Dallas, and The University of Texas Health Science Center at San Antonio. All sites collected data using protocol-specific case report forms (CRFs), which were developed from the standard set of DCP Chemoprevention CRF templates utilizing the National Cancer Institute – approved Common Data Elements. Also, all sites reported clinical data using the DCP Oracle clinical remote data capture Web-based application managed by DCP's monitoring contractor (see detailed study information at: https://clinicaltrials.gov/ct2/show/NCT00438464?term=finasteride&rank=8).

2.2. Study Participants

The Pocock-Simon Minimization Method (Pocock & Simon, 1975), a dynamic randomization method, was used to randomize patients and stratify them by biopsy GS (6 versus 7), type of prostatectomy (open vs. laparoscopic/robotic), and study site. Developed at the lead organization and available by intranet, a software program organized the randomization of participants and the dispensing of the study drug at the initial clinical evaluation. Neither patients nor medical staff members were aware of assignment, and placebo and finasteride pills were matched in appearance.

Eligible patients had histologic proof of clinically organ-confined adenocarcinoma of the prostate, clinical stage T1c or T2 disease with GS 6(3 + 3) or 7(3 + 4) or 7(4 + 3), and a prostate-specific antigen (PSA) value <10 ng/mL before registration. Participants agreed while on study not to take dehydroepiandrosterone, phytoestrogen supplements, antiandrogen therapy, saw palmetto, or dutasteride or finasteride pills independent of those provided by the study. They also had to have an Eastern Cooperative Oncology Group performance status of ≤2, be scheduled for prostatectomy in 4–6 weeks, and agree to use adequate contraception before and throughout the study. Exclusion criteria included active malignancy at any other site; prior radiation therapy for the primary tumor; history of allergic reactions attributed to compounds of chemical or biologic composition similar to that of finasteride; uncontrolled intercurrent illness; use of anticoagulation agents, except for cardioprotective doses of aspirin; use of all hormonal agents, including testosterone, saw palmetto, dutasteride, or finasteride six months before study entry (see detailed study information at: https://clinicaltrials.gov/ct2/show/NCT00438464?term=finasteride&rank=8).

Primary and secondary end points were prespecified and were assessed after prostatectomy. The primary end point was to compare the frequency of the expression of the predetermined molecular signature (ERβ, UBE2C, SRD5A2, and VEGF) differentiating high- and low-grade tumors in the GG3 areas of the two study groups, adjusted for GS at prostatectomy. The secondary end points were to compare the

frequency with which GG3 and GG4 tumor occurs in the prostatectomy specimens in the two study groups, and to compare the frequency of the expression of the predetermined molecular signature in the GG4 areas of the two study groups, adjusted for GS at prostatectomy. We also compared AR levels, Ki-67, and cleaved caspase 3 across grades between and within treatment groups.

2.3. Clinical Intervention

Patients were randomly assigned to take a 5-mg finasteride tablet or matching placebo by mouth daily for 4–6 weeks until admitted for prostatectomy. Adherence to the regimen was evaluated by pill diary and a count of leftover tablets.

2.4. Specimen Interrogation

2.4.1. Laboratory Investigation

PSA, testosterone, DHT, estrone, and estradiol levels were measured at baseline and 24–96 h before prostatectomy. The blood specimens were processed per a standard operating procedure (Supplementary Methods S1). Within 24 h of collection, investigators froze and shipped specimens to the lead organization according to guidelines outlined in the International Air Transport Association *Dangerous Goods Regulations* (International Air Transport Association, 2006). Levels were measured by Quest Diagnostics.

2.4.2. Pathology

Pathologists from the four institutions were blinded to treatment and agreed prospectively on standard operating procedures for handling prostatectomy specimens (Supplementary Methods S2) and processing tissue specimens (Supplementary Methods S3). In brief, prostatectomy specimens were received in ice from the operating room. Three sets of tissues by the following biopsy scheme were collected (for future research): sextant biopsy specimens from the posterior surface, left and right transition zone, one core from each from the anterior surface, and palpable nodules, if present (Supplementary Methods S2). One set was placed in formalin and processed to paraffin; another was placed in RNAase for 24 h at room temperature and then embedded and frozen in OCT; and a third set was frozen in OCT and archived at −80 °C. After biopsy cores were collected, the prostatectomy specimen was inked, placed in 10% neutral buffered formalin, fixed for a minimum of two days, and grossed for patient care histologic procedures and diagnostics.

2.4.3. Immunohistochemistry

Sections of formalin-fixed paraffin-embedded prostatectomy specimens stained with hematoxylin and eosin (HE) prospectively collected were reviewed. In order to minimize biases that might be introduced because of tumor heterogeneity, the study required that the dominant tumor focus in the peripheral zone of the prostatectomy specimens be used for molecular marker analysis. The revised Gleason scoring system (Epstein et al., 2005) was used for pathological evaluation.

Cases were selected for immunohistochemistry based on (*a*) presence of acinar prostatic adenocarcinoma exclusive of other histologic types; (*b*) presence of at least one tumor originating in the peripheral zone of the prostate; (*c*) GS of 6 (3 + 3), 7 (3 + 4), or 7(4 + 3); (*d*) presence of GG3 and/or GG4 forming distinct clusters; (*e*) tissue preservation; and (*f*) size of at least 0.5 cm in greatest dimension within a section. Criteria for exclusion were (*a*) presence of carcinoma of the prostate other than acinar; (*b*) absence of adenocarcinoma originating in the peripheral zone; (*c*) GS of 8 or above; (*d*) insufficient tumor in recuts; and (*e*) poor tissue preservation. Presence of tertiary GG5 was not a criterion for exclusion of the case. Particular sections of any given case that had been decalcified prior to paraffin embedding were excluded. One block per case that best represented distinct areas of GG3 and/or

GG4 was selected and retrieved from the archive. Consecutive blank sections of the block were prepared on charged slides for immunohistochemistry, and the last of those sections was stained with HE.

Areas of interest were selected under light microscopy on HE sections obtained prior to and after sections were cut for markers to check for sufficient tumor through all the stained sections (Eclipse CiL, Nikon). Five or more GG3 or GG4 glands within an area of analysis from the largest peripheral zone focus were evaluated per specimen. Consensual diagnostic criteria were used to identify GG3 and GG4 (fused glands or poorly formed glands). Different GG4 patterns, including fused, poorly formed, and cribriform, were scored, but only the predominant pattern was included in the analysis. Cribriform was the predominant GG4 pattern in only seven cases (two in the finasteride arm and five in the placebo arm). A permanent marker was used to map the areas on the slides. Five or more GG3/GG4 glands within an area of analysis from the dominant cancers in the peripheral zone were evaluated per specimen. Using these specimens, we undertook discriminating marker expression studies to detect seven markers: Ki67, AR, and cleaved caspase 3 (BOND-III automated immunostainer, Leica Biosystems); and estrogen receptor β (ERβ), ubiquitin-conjugating enzyme E2C (UBE2C), 3-oxo-5α-steroid 4-dehydrogenase 2 (SRD5A2), and vascular epithelial growth factor (VEGF) (Dako autostainer, Dako North America). (See Supplementary Methods S4—Procedure for Derivation and Analysis of Preselected Molecular Markers.)

Immunohistochemistry study was performed in the Stanford Alexander Tissue Derivative Laboratory (by EE) of the David H. Koch Center for Applied Research of Genitourinary Cancers at MD Anderson Cancer Center. Multitissue controls were used. Incubation was with primary antibodies Ki-67 (1:100; clone MIB-1, Dako), AR (1:30; clone AR441, Dako), cleaved caspase 3 (1:200; polyclonal, cell signaling technology), ERβ (1:15; clone PPG5/10, Dako; positive controls, breast and endometrial cancers; negative controls, lung and colon), UBE2C (1:50; clone 9D3, Novus Biologicals; positive control, placenta), SRD5A2 (1:400; polyclonal, LifeSpan BioScience; positive control, testis; negative controls, colon and lung), and VEGF (1:1; clone SP28, Abcam; positive control, tonsil). A 3,3′-diaminobenzidine chromogen was used on all biomarkers.

The HE and immunostained slides for Ki-67, AR, cleaved caspase 3, ERβ, and UBE2C were scanned (Aperio ScanScope XT, Aperio Technologies). Areas of interest were selected on virtual immunostained slides by a pathologist (EMLNT) using mapped HE slides as reference (ImageScope, Aperio Technologies). A nuclear algorithm was used to measure the percentage of immunoreactive cells (nuclear v9 algorithm, Aperio Technologies) for Ki-67, AR, ERβ, and UBE2C, and a cytoplasmic algorithm (Color Deconvolution v9) was used for cleaved caspase 3. Each area of interest produced individual Excel files, including the percentage of positive cells.

Because of technical limitations, markers with diffuse cytoplasmic staining (SRD5A2 and VEGF) were manually quantitated. The percentage of reactive epithelial cells respective of the total number of carcinoma cells of the area was recorded on a 0–100% scale. Intensity of staining and the subcellular localization were noted.

2.4.4. Statistical Analysis

The prespecified primary objective was to compare discriminating molecular marker expression in GG3 areas of the finasteride group with those of the placebo group, adjusting for GS at prostatectomy. A predefined grade-associated molecular signature was designed and analyzed before use in tissue interrogation (Supplementary Methods S4). Sample size was determined by a power analysis that dictated having 100 in each group to provide 80% power to detect an effect size of 0.41 in biomarker expression between the two groups, at a two-sided significance level of 0.05. Early stopping rules and inclusion/exclusion criteria were prospectively established, and the protocol required an interim analysis after the first half of patients

were enrolled to guard against waste of resources if results were overwhelmingly positive or negative. Patients remained on the study until the protocol intervention was completed with prostatectomy. All data were collected at the sites and analyzed centrally. We used descriptive statistics to summarize patient characteristics by arm and Fisher's exact test and the Wilcoxon rank-sum test to assess differences between the arms. All testing was two sided, unless reported otherwise.

We also used the Wilcoxon rank-sum test to assess differences in molecular markers (four components of the predefined grade-associated molecular signature [ERβ, UBE2C, VEGF, and SRD5A2] and three markers [Ki-67, cleaved caspase 3, and AR] added post hoc to the analysis) between and within the two arms. Ki-67 and cleaved caspase 3 were studied to assess the effect of finasteride on proliferation and apoptosis, and AR was studied because it lies downstream of 5α-reductase, a target of finasteride, and is central to prostate cancer development and progression. The Wilcoxon signed-rank test was used to assess the within-arm changes of each of these markers.

To assess exploratory end points, we fitted a multivariable logistic regression model with the four biomarkers of the predefined grade-associated molecular signature, age, and exposure (finasteride versus placebo) as covariates. Potential interaction effects between biomarkers and exposure arm were explored. Similar analyses assessed the effect of AR, considering the potential interaction between AR and the exposure arm. For analyses of both the predefined and the current markers, no formal adjustments for multiple comparisons were made because of the analyses' exploratory nature. All statistical analyses were performed using SAS 9.3.

3. Funding and Nonfinancial Support

4. Results

4.1. Study Population

Of 2761 patients screened, 204 were randomized between February 2007 and March 2012, and 183 (89.7%) received treatment and were evaluable (Fig. 1). The median treatment for both groups was 28 days. Men assigned to the finasteride arm were younger than those in the placebo arm (median age, 59 years [range, 45–73] versus 62 years [range, 48–73]; P = 0.005); otherwise, the two arms were well matched (Supplementary Table S1). As measured by pill diary and pill count, adherence was 97.8%. No participant experienced an adverse event of greater than grade 2 toxicity (National Cancer Institute, 2006).

Similarly, the two groups providing prostatectomy specimens were balanced for clinical characteristics except for age (Supplementary Table S2). The finasteride group was younger than the placebo group (median age, 58 years [range, 45–73] versus 63 years [range, 48–73];
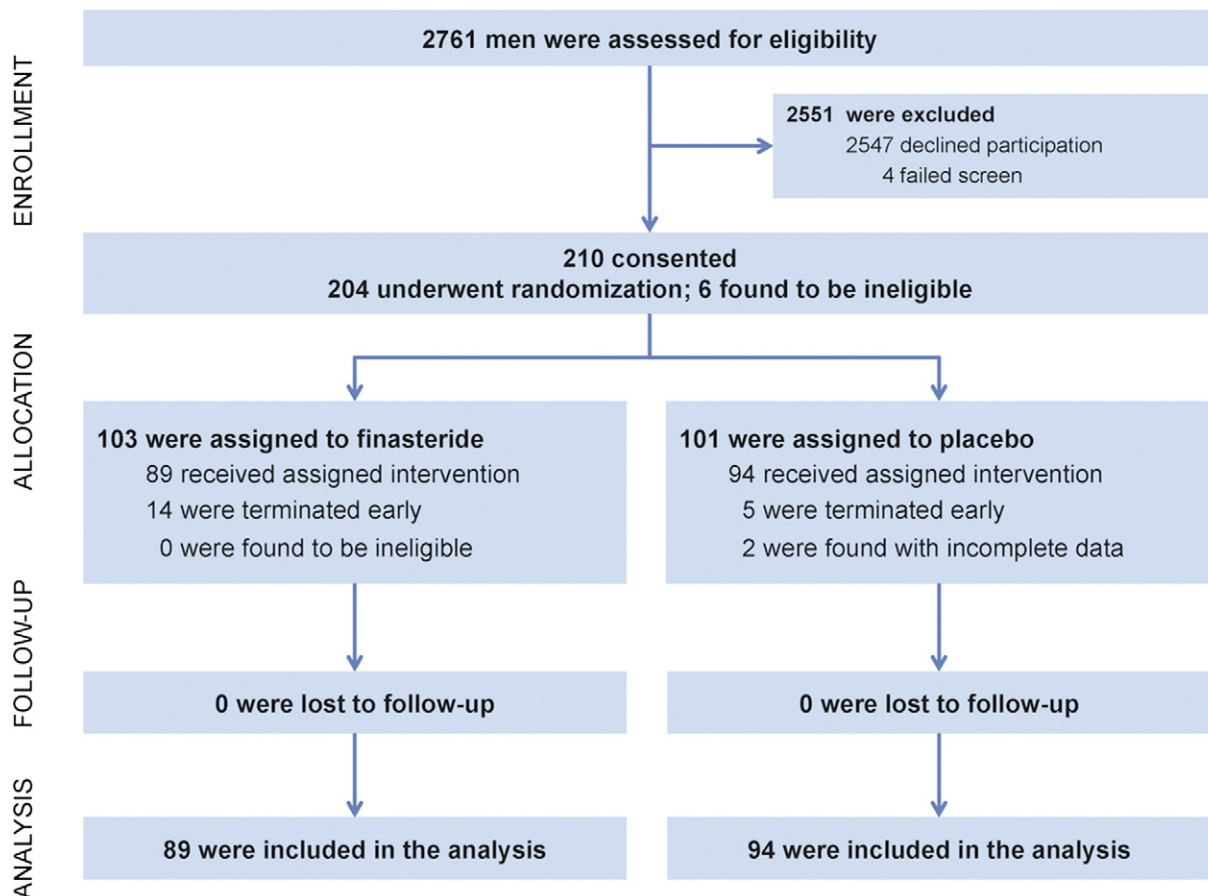

Fig. 1. Enrollment and subject disposition. Participants were enrolled in the study at four sites from February 2007 to March 2012.

P = 0.004). One hundred thirty prostatectomy specimens were available for molecular marker analysis. In the finasteride arm and placebo arm, there were 67 and 75 GG3 tumor areas and 57 and 61 GG4 tumor areas, respectively.

4.2. Pathologic Outcomes

Pathologic characterization of the prostatectomy specimens showed no difference between the groups (Table 1), which was also true for the biomarker study groups (Supplementary Table S3). Patient tumor volume median values were similar overall (finasteride, 1.0 mL [range, 0–9.3 mL]; placebo, 0.8 mL [range, 0–10.4 mL]; P = 0.73), as were values for cancer foci in the peripheral zone (finasteride, 0.6 mL [range, 0–9.3 mL]; placebo, 0.5 mL [range, 0–9 mL]; P = 0.75) and the transition zone (finasteride, 0.0 mL [range, 0–6 mL]; placebo, 0.0 mL [range, 0–10.4 mL]; P = 0.84). Likewise, tumor volume median values for the biomarker subgroup were similar overall (finasteride, 0.9 mL [range, 0.01–7.8 mL]; placebo, 0.8 mL [range, 0.01–5.5 mL]; P = 0.79), as were values for cancer foci in the peripheral zone (finasteride, 0.6 mL [range, 0.01–7.8 mL]; placebo, 0.7 mL [range, 0–4.2 mL]; P = 0.40) and the transition zone (finasteride, 0.03 mL [range, 0–5.8 mL]; placebo,

0 mL [range, 0–3.6 mL]; P = 0.43). After short-term exposure, no significant differences were found between the groups in GS upgrading between biopsy and radical prostatectomy, in tumor volume, or in number of cancer foci. The important premise of this study was that there would be no morphologic changes after brief exposure to finasteride so that molecular changes could be delinked from morphology.

4.3. PSA and Hormone Levels

Before intervention, PSA and serum steroid hormone levels were not statistically different between groups; after intervention, the finasteride group had significantly lower PSA (3.2 ng/mL versus 5.2 ng/mL; P < 0.001) and DHT levels (10 ng/dL versus 27 ng/dL; P < 0.001) and a significantly higher testosterone level (382 ng/dL versus 327 ng/dL; P = 0.04) than the placebo group. The percentage change was also significantly different between the groups (Supplementary Table S4). Estrone and estradiol levels were similar. The same significant asymmetric distribution in the posttreatment levels of PSA, testosterone, and DHT between the groups was observed in the biomarker study (Supplementary Table S5).

4.4. Molecular Markers

In the areas scanned for markers Ki-67, AR, ERβ, and UBE2C, we found no significant differences between the two groups in either tumor grade, and the same was true for individual components. Due to the heterogeneity of the tumor areas, the correlation in molecular marker expression between different tumor areas was deemed very minimal. Box plots were generated to display the distribution of the area analyzed and molecular biomarkers by treatment group and GG (see Supplementary Figs. S1 and S2).

The individual components of the predefined grade-associated molecular signature and Ki-67, AR, and cleaved caspase 3 were compared between and within groups. Because our predefined molecular signature could not distinguish GG4 from GG3 tumor areas in the placebo arm, we were unable to address the primary objective. However, although no significant difference was found in GG3 tumors between the groups (median, 75.2% versus 78.3%, P = 0.41) (Table 2; Supplementary Fig. S2A), the level of AR in finasteride-exposed GG4 tumors was significantly lower than that in placebo group GG4 tumors (median, 63.7% versus 75.9%, P = 0.04) (Supplementary Fig. S2B). Within the finasteride group, AR expression in GG4 tumor areas was numerically lower than it was in GG3 tumor areas (median, 63.7% versus 75.2%, P = 0.09) (Table 3; Supplementary Fig. S2C). By comparison, the cleaved caspase 3 level was significantly higher in GG3 (median, 0.2% versus 0.08%, P = 0.03) and GG4 (median, 0.06% versus 0.04%, P = 0.02) tumors from the finasteride group than in those from the placebo group (Table 3; Supplementary Figs. 2A and 2B). Within each arm, the cleaved caspase 3 level was significantly higher in GG3 tumors than in GG4 tumors (finasteride: 0.2% versus 0.06%, P < 0.001; placebo: 0.08% versus 0.04%, P < 0.001) (Table 3; Supplementary Figs. 2C and 2D).

In the post hoc analysis of the predefined grade-associated molecular signature, the fitted multivariable logistic regression model suggested no significant association between GG and the biomarkers. Additionally, no significant interaction was found between each biomarker and the exposure, and the fitted model indicated neither significant association with GG nor significant interaction with exposure. Screening for potential confounders ruled out the possibility that the age difference between the groups was responsible for the study's results.

5. Discussion

Our results provide insight into grade-specific effects of 5α-reductase inhibition in early prostate cancer. AR expression was significantly lower in the finasteride group than in the placebo group and

Table 1
Pathologic characteristics of radical prostatectomy specimens after treatment: All evaluable patients.

Characteristics	Finasteride (N = 89)		Placebo (N = 94)		P
	N	(%)	N	(%)	
Gleason score					
6	12	(13.6)	10	(10.8)	
7	71	(80.7)	78	(83.9)	
8	2	(2.3)	1	(1.1)	0.84
9	3	(3.4)	4	(4.3)	
Gleason grade—specimen (primary)					
3	65	(73.9)	75	(80.6)	
4	23	(26.1)	18	(19.4)	0.29
Gleason grade—specimen (secondary)					
3	31	(35.2)	24	(25.8)	
4	53	(60.2)	64	(68.8)	0.37
5	4	(4.5)	5	(5.4)	
Tumor, node, metastasis stage					
pT2	73	(82.0)	74	(78.7)	
pT3a	11	(12.4)	15	(16.0)	0.81
pT3b	5	(5.6)	5	(5.3)	
Margin of resection					
Positive	17	(19.1)	16	(17.0)	
Negative	67	(75.3)	76	(80.9)	0.40
Equivocal	5	(5.6)	2	(2.1)	
Lymph node status					
pN0	46	(51.7)	43	(45.7)	
pN1	2	(2.2)	2	(2.1)	0.79
pNX	41	(46.1)	49	(52.1)	
Cancer foci					
1	15	(16.9)	11	(11.7)	
2	18	(20.2)	20	(21.3)	
3	28	(31.5)	28	(29.8)	0.73
4	13	(14.6)	20	(21.3)	
≥5	15	(16.8)	15	(15.9)	
Zonal origin of tumor foci per radical prostatectomy specimen					
PZ	35	(39.3)	38	(40.4)	
PZ + TZ	49	(55.1)	50	(53.2)	
PZ + TZ + CZ	2	(2.2)	2	(2.1)	0.99
PZ + CZ	0	(0.0)	1	(1.1)	
TZ	3	(3.4)	3	(3.2)	
Zonal origin—dominant tumor focus					
PZ	67	(75.3)	73	(77.7)	
TZ	22	(24.7)	21	(22.3)	0.73
Gleason upgrade between biopsy and prostatectomy					
No	63	(70.8)	70	(74.5)	
Yes	26	(29.2)	24	(25.5)	0.62

Abbreviations: PZ, peripheral zone; TZ, transition zone; CZ, central zone.

Table 2
Comparison of biomarkers between treatment arms: Biomarker subgroup.

Biomarkers	Within Gleason grade 3				Within Gleason grade 4			
	N	Mean ± SD	Median (range)	P	N	Mean ± SD	Median (range)	P
VEGF3								
Finasteride	37	63.9 ± 36.3	80 (5–100)	0.70	48	63.6 ± 35.5	85 (5–100)	0.45
Placebo	55	63.7 ± 38	90 (0–100)		61	59.8 ± 35.4	70 (0–100)	
ERβ								
Finasteride	35	18.1 ± 15.6	15.0 (0.03–59.0)	0.38	48	15.0 ± 15.1	8.0 (0–49.6)	0.83
Placebo	55	14.8 ± 14.7	6.6 (0.01–56.3)		62	16.7 ± 18.8	9.5 (0–72.8)	
AR								
Finasteride	35	69.8 ± 17.3	75.2 (21.5–92.1)	0.41	48	64.3 ± 16.67	63.71 (13.5–92.0)	0.04
Placebo	54	72.4 ± 16.7	78.3 (33.8–97.6)		62	68.6 ± 23.1	75.9 (1.4–96.6)	
Ki-67								
Finasteride	37	1.6 ± 1.4	1.1 (0.05–5.4)	0.75	48	1.8 ± 1.6	1.3 (0.05–7.2)	0.80
Placebo	54	1.5 ± 1.0	1.3 (0.03–4.5)		62	1.7 ± 1.4	1.4 (0.05–7.3)	
SRD5A2								
Finasteride	45	72.9 ± 37.0	100 (0–100)	0.57	38	71.8 ± 37.6	95 (0–100)	0.61
Placebo	47	64.7 ± 43.7	90 (0–100)		69	64.9 ± 40.8	90 (0–100)	
UBE2C								
Finasteride	34	0.5 ± 0.4	0.4 (0–1.5)	0.12	46	0.5 ± 0.4	0.3 (0–1.6)	0.86
Placebo	55	0.5 ± 0.5	0.3 (0–2.4)		62	0.5 ± 0.4	0.3 (0–2.3)	
Caspase								
Finasteride	38	0.4 ± 0.7	0.2 (0.01–4.1)	0.03	47	0.1 ± 0.1	0.06 (0–0.5)	0.02
Placebo	55	0.2 ± 0.2	0.08 (0–0.8)		61	0.06 ± 0.08	0.04 (0–0.6)	

Abbreviations: VEGF, vascular epithelial growth factor; ERβ, estrogen receptor beta; AR, androgen receptor; SRD5A2, 3-oxo-5α-steroid 4-dehydrogenase 2; UBE2C, ubiquitin-conjugating enzyme E2C.

numerically lower in GG4 than GG3 areas within the finasteride group. Expression of cleaved caspase 3, an indicator of apoptosis, while significantly higher in the finasteride group than the placebo group in both GG3 and GG4 areas, was found to be significantly lower in GG4 areas in both groups. The molecular alterations in AR signaling, an adaptive response to finasteride, were observed only in the GG4 pattern, evidence of the inherent survival advantage indicated by lower levels of cleaved caspase 3 in GG4 than in GG3 tumor areas in both groups.

This type of difference in cell proliferation was also observed in a study by Kosaka et al. (2014), who found that 5α-reductase inhibitors (5ARIs) reduced an indicator of cell proliferation—CDC6 mRNA—in the androgen-dependent LNCaP cell line but saw the proliferation marker increase in androgen-independent lines C4-2 and C4-2AT6, the last a castration-resistant prostate cancer. Interestingly, they found the

reverse effect with DHT. Kosaka and colleagues concluded that 5ARIs might help accelerate adaptation to aggressive phenotypes in some prostate cancer types.

Finally, our finding of lower AR expression in GG4 tumor areas after short-term finasteride exposure is in line with an emerging concept that reduced androgens in prostate tissues may over time lead to derepression of AR expression, which in turn deregulates AR function and downstream derepression of specific AR target genes normally repressed by androgens (Cai et al., 2011). These gene activities may lead to stochastic activation of oncogenic signaling that promotes the development of aggressive prostate cancer.

Molecular modulations we detected suggest temporal dependence and on-target effects of finasteride are grade dependent, which may account for previously reported selective suppression by finasteride of

Table 3
Comparison of biomarkers between GG3 and GG4: Biomarker subgroup.

Biomarker	Within finasteride arm				Within placebo arm			
	N	Mean ± SD	Median (range)	P	N	Mean ± SD	Median (range)	P
VEGF								
GG3	37	63.9 ± 36.3	80 (5–100)	0.84	55	63.7 ± 38	90 (0–100)	0.32
GG4	48	63.7 ± 35.5	85 (5–100)		61	59.7 ± 35.4	70 (0–100)	
ERβ								
GG3	35	18.1 ± 15.6	15.0 (0.03–58.9)	0.36	55	14.8 ± 14.7	6.6 (0–56.3)	0.83
GG4	48	15.0 ± 15.1	8.0 (0–49.56)		62	16.7 ± 18.8	9.5 (0–72.8)	
AR								
GG3	35	69.8 ± 17.3	75.2 (21.5–92.1)	0.09	54	72.4 ± 16.7	78.3 (33.77–97.6)	0.77
GG4	48	64.3 ± 16.7	63.7 (13.5–92.0)		62	68.6 ± 23.1	75.9 (1.39–96.6)	
Ki-67								
GG3	37	1.6 ± 1.4	1.1 (0.05–5.4)	0.46	54	1.5 ± 1.0	1.3 (0.03–4.5)	0.87
GG4	48	1.8 ± 1.6	1.3 (0.05–7.2)		62	1.7 ± 1.4	1.4 (0.05–7.3)	
SRD5A2								
GG3	45	72.9 ± 37.0	100 (0–100)	0.88	47	64.7 ± 43.7	90 (0–100)	0.91
GG4	38	71.8 ± 37.6	95 (0–100)		69	64.9 ± 40.8	90 (0–100)	
UBE2C								
GG3	34	0.5 ± 0.4	0.4 (0–1.5)	0.18	55	0.5 ± 0.5	0.3 (0–2.4)	0.90
GG4	46	0.5 ± 0.4	0.3 (0–1.6)		62	0.5 ± 0.4	0.3 (0–2.3)	
Caspase								
GG3	38	0.4 ± 0.7	0.2 (0.01–4.1)	<0.001	55	0.2 ± 0.2	0.08 (0–0.8)	<0.001
GG4	47	0.1 ± 0.1	0.06 (0–0.5)		61	0.06 ± 0.08	0.04 (0–0.6)	

Abbreviations: VEGF, vascular epithelial growth factor; GG, Gleason grade; ERβ, estrogen receptor beta; AR, androgen receptor; SRD5A2, 3-oxo-5α-steroid 4-dehydrogenase 2; UBE2C, ubiquitin-conjugating enzyme E2C.
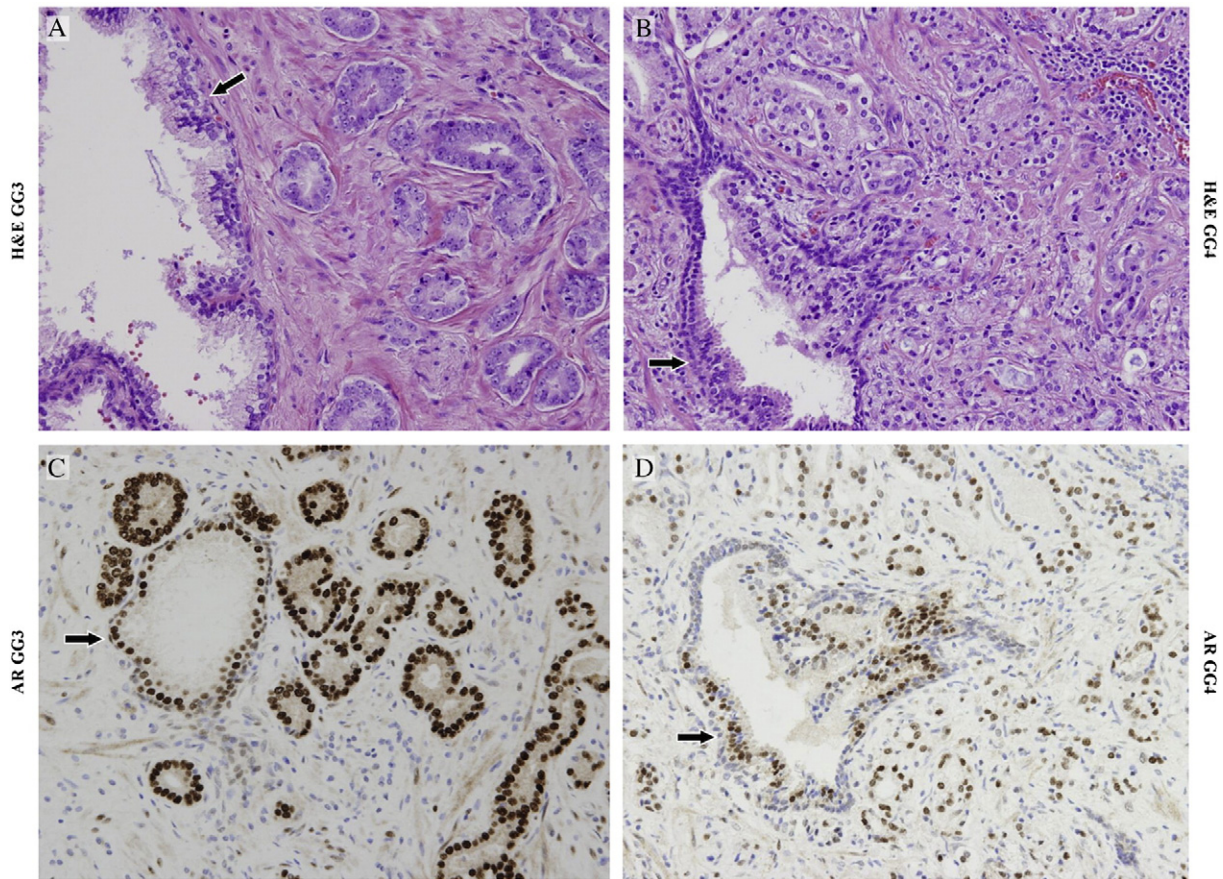
Fig. 2. Comparisons of finasteride-exposed Gleason grade 3 and 4 tumors. Pathology studies of finasteride-exposed group Gleason grade (GG) 3 component are compared with those of the GG4 component. Hematoxylin and eosin stains of GG3 and GG4 tumor areas (A and B, respectively) from two different cases and corresponding parallel illustrations of immunohistochemistry for androgen receptor (C and D, respectively) are shown. Black arrows indicate nonneoplastic glands.

low-grade tumors. Reasons the prespecified molecular signature was not detected may include the discrepancy between the source of tissues used to define the signature and the early cancer cases in the study. Specimens used to define the signature were derived from more extreme samples of prostate cancer (GG3 specimens from GS6 prostate cancer and GG4 specimens from GS8 or GS9 prostate cancers, including all GG4 patterns) than the generally low- or intermediate-risk cancers present in the study population. Therefore, in subsequent studies, investigators may need to match more closely entry criteria of men who are subjects and characteristics of men from whom tissue samples are taken.

The development of the prespecified molecular signature had been based on the hypothesis that if finasteride caused high-grade cancer, a high-grade molecular signature would be detectable with greater frequency after exposure to finasteride than after exposure to placebo. This was based on the presumption that disease progression in early stage prostate cancer occurred in a step-wise fashion (e.g., GG3 tumors would precede GG4 tumors), but progression theories remain controversial.

Wang et al. (2009), for example, have shown in mice that castration-resistant Nkx3-1 (CARN) cells—rare luminal epithelial stem cells that survive androgen withdrawal—can give rise to basal, luminal, and neuroendocrine cells after restoration of androgen. Others have shown these cells also regenerate ducts and self-renew (Leong et al., 2008). By deleting the tumor-suppressing gene *Pten* in these cells, Wang et al. further demonstrated that high-grade prostatic intraepithelial neoplasia, and carcinoma rapidly formed in the *Pten*-free system, with CARN cells serving as cells of origin after androgen-mediated renewal. It wasn't that we expected, based on the long-standing presumption of step-wise progression, that finasteride would be found responsible

for the high-grade disease detected by others; in fact, it was an effort to examine the nexus of transition and begin to characterize the molecular processes.

Our study's design provided an ideal opportunity to back-validate markers of apoptosis (e.g., cleaved caspase 3) and cellular proliferation (e.g., Ki-67), which are commonly used in this class of chemopreventive agents as surrogates for chemoprevention efficacy in short-term, phase II clinical trials in prostate cancer and other cancers (Parnes et al., 2013). After short-term exposure to finasteride, expression of the apoptotic factor cleaved caspase 3 in GG3 and GG4 tumor areas was significantly increased, demonstrating, as shown in the PCPT, preventive efficacy. Additionally, within both arms, levels of cleaved caspase 3 were significantly lower in GG4 tumors than in GG3 tumors. Ananthanarayanan et al. (2006) and Ummanni et al. (2010) independently found that, compared with levels of activated caspase 3 in normal prostate epithelium, levels of activated caspase 3 in untreated prostate cancer from radical prostatectomy specimens were significantly lower. Evidence that Ki-67 was modulated by finasteride was not detected during the study.

Findings conflict regarding the influence of 5ARIs on apoptosis. Bass et al. (2009) showed no effect on caspase 3 in a randomized, placebo-controlled, presurgical 30-day finasteride intervention in localized prostate cancer ($N = 22$ in each arm). However, results of two dutasteride studies, both using terminal deoxynucleotidyl transferase dUTP nicked-end labeling, indicated effects, but findings were contradictory. Andriole et al. (2004), in a double-blind, randomized placebo-controlled trial, reported higher apoptosis levels in 46 men with clinically organ-confined prostate cancer treated with dutasteride or placebo for 6–10 weeks before prostatectomy. In contrast, Gleave et al. (2006) reported a significant decrease in apoptosis when dutasteride was

compared with placebo for four months before prostatectomy. This further supports the notion that the molecular effects of 5ARIs depend on exposure duration.

In contrast to our results, findings of Thomas et al. (2008) in untreated tumors indicate expression of SRD5A2, the target of finasteride, is higher in high-grade than in low-grade localized disease. Their earlier work had indicated in part that SRD5A2 expression rose as prostate cancer progressed (Thomas et al., 2005). Differences between study populations may explain the discordance, inasmuch as Thomas et al. examined GG3, GG4, and GG5 components from cancer overall (GSs of 5–8, GSs of 7–9, and GSs of 8–10, respectively), whereas expression we studied was in tumor foci with a narrow range of Gleason scores.

Although we detected no change in the estrone or estradiol ligands of ER, estrogen has been shown by others to be important in prostate carcinogenesis and progression (Weihua et al., 2002). In early prostate cancer in the context of prostate cancer prevention with finasteride (Mak et al., 2010), 3β-adiol, a metabolite of DHT and a ligand of ERβ, may be important in cross-talk between AR and ER signaling. Furthermore, the biological significance of ER signaling in persistent low- and high-grade cancers warrants further investigation.

Limitations of this study and its findings include the brief period over which the study was conducted and the focus on tumors of the peripheral zone. We did not observe an interaction between finasteride and ERβ or between finasteride and SRD5A2 in comparisons by group or GG; however, it may be that molecular adaptation by tumors to finasteride exposure may occur over a longer period of time than was assessed in this investigation. Likewise, Ki-67's failure to be modulated by finasteride, a drug proven to reduce prostate cancer risk, underscores the time limitation in this trial. In addition, it is a limitation that our results are based on tumors of peripheral zone origin and that the GG patterns evaluated primarily included poorly formed and fused glands. Therefore, it remains unknown if our results apply to tumors of transition zone origin or other GG4 patterns.

Ours is one of few studies in early prostate cancer to consider the time it takes for response to therapy to be reflected in gene expression, but it has been studied in more advanced prostate cancers. Although AR expression four months following androgen deprivation therapy in a neoadjuvant setting before radical prostatectomy was not increased in hormone-naive high-grade cancers (Efstathiou et al., 2013), an increase in expression and a commensurate AR gene copy number increase have been frequently observed in castration-resistant prostate cancer (Scher et al., 2004), and expression further increased after only eight weeks of abiraterone (Efstathiou et al., 2012). Our findings emphasize the need to understand the temporal relationships of finasteride-induced molecular changes and their biological and clinical implications.

Overall, finasteride's differential modulation of AR in GG3 versus GG4 areas of tumor suggests grade-associated differences in AR signaling in early prostate cancer. The heterogeneity of AR signaling networks has been recognized for a long time. In work with cell lines (Li et al., 2011), we found dramatic variability characterized AR signaling: complicated self-regulation controlled the central axis of AR signaling, 5α-reductase expression varied across cell lines, each cell line varied in response to androgen, androgen controlled 5α-reductase transcription through the AR pathway, and expression of each of the three 5α-reductase enzymes was cell-type specific.

Confining the study to the largest focus of the peripheral zone, thereby limiting any bias introduced by the tumor heterogeneity inherent in the multifocal and multizonal nature of prostate cancer, reinforces confidence in the findings. Perhaps high-grade tumors are more sensitive than low-grade tumors to the disequilibrium of testosterone and DHT caused by finasteride or dutasteride, and, as a consequence, AR expression paradoxically decreases as a short-term adaptation. A corollary may be that low-grade tumors are more dependent than high-grade tumors on DHT. If so, this may be the mechanism whereby finasteride and dutasteride reduced the likelihood of finding low-grade tumors on biopsy in other trials (Thompson et al., 2003; Andriole et al., 2010).

In summary, this randomized, controlled trial, which was undertaken to improve understanding of molecular modulations associated with high-grade disease found in finasteride-exposed clinically organ-confined prostate cancer, identified grade-specific AR molecular effects that refine notions of appropriate use of 5ARIs and suggest specialized applications. Confirmation of these findings may provide a dynamic test enabling prospective identification of finasteride-responsive cancers or lead to predictive markers for allocating therapies or predicting risk of progression.

Authors' Contributions

Conception and design: J. Kim, H. L. Parnes, C. J. Logothetis, P. Troncoso.

Acquisition or interpretation of data: All authors did one or the other or both.

Analysis and interpretation of data: X. Wang, K.-A. Do, J. J. Lee.

Writing, reviewing, and/or revising the manuscript: All authors.

Approving the final manuscript: All authors.

Study supervision: J. Kim.

Conflict of Interest

Jeri Kim, MD, acknowledges receipt of nonfinancial support from Merck, which provided 5-mg tablets of finasteride and matching placebos for the study. For the 3 years preceding submission, she has no other conflict of interest to report. Ian Thompson, MD, has received finasteride and matching placebo from Merck for research. No author has any patents—planned, pending, or issued—relevant to the work. No other conflict of interest is reported.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.03.047.

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