The Effects of the Dual 5α-Reductase Inhibitor Dutasteride on Localized Prostate Cancer—Results From a 4-Month Pre-Radical Prostatectomy Study

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BACKGROUND. As dihydrotestosterone (DHT) is the most potent androgen in the prostate, inhibition of the 5α-reductase isoenzymes, which convert testosterone to DHT, could be an appropriate target for the treatment of prostate cancer.
METHODS. Eighty-one men with clinically localized prostate cancer received daily dutasteride 3.5 or 0.5 mg, or no therapy for 4 months before radical prostatectomy. Histopathological assessments were conducted on prostatectomy specimens.
RESULTS. Treatment with dutasteride was associated with reductions in serum and intraprostatic DHT of $\geq 90\%$, and a decrease in total prostate and tumor volumes. No effect of dutasteride was noted on Gleason grade. Histopathological effects on benign tissue were similar but less prominent than those seen with androgen ablation, whereas there was no significant difference in cancer histology among the groups.
CONCLUSIONS. Dutasteride treatment results in similar but less marked changes compared with androgen ablation. *Prostate 66: 1674–1685, 2006.* © 2006 Wiley-Liss, Inc.

KEY WORDS: 5 alpha reductase; prostate cancer; treatment; Gleason score; apoptosis; proliferation

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INTRODUCTION

From the late nineteenth century it has been hypothesized that 'testicular factors' promoted benign prostatic growth [1]. In the early 1940s, serum acid phosphatase levels were demonstrated to be decreased by castration and increased by androgens [2], and castration was shown to result in clinical and serological improvements in men with prostate cancer [3]. Clinical studies have since established androgen ablation as a key component of prostate cancer management. Given the knowledge that dihydrotestosterone (DHT) and not testosterone is the most potent androgen in the prostate [4], inhibition of the 5α-reductase isoenzymes could be an appropriate target for the treatment of prostate cancer.

A number of lines of evidence support this hypothesis. Firstly, studies with 5α-reductase inhibitors have demonstrated that they inhibit proliferation of human LNCaP and PC-82 prostate cancer cells in vitro [5,6], as well as tumor growth in the Dunning rat model [7–9]. Secondly, several small-scale studies of finasteride in men with advanced prostate cancer demonstrated serological improvements, albeit without evidence of tumor regression or prevention of recurrence [10,11]. Lastly, the Prostate Cancer Prevention Trial (PCPT) demonstrated that daily therapy with finasteride significantly reduced the prevalence of prostate cancer versus placebo over a 7-year period [12]. Given that the rates of prostate cancer in men treated with finasteride and placebo diverged early in the study, it seems plausible that finasteride treated sub-clinical, microscopic tumors that were not clinically apparent at baseline [12].

If 5α-reductase inhibitors confer a treatment benefit in prostate cancer, an in vitro effect at the histological, as well as clinical level, would be expected. A number of studies have examined the effects of 5α-reductase inhibitors on the histology of the benign and hyperplastic human prostate [13], but data on their effects in prostate cancer are less comprehensive. For the Type 2-selective agent finasteride, a single needle biopsy study failed to demonstrate any effect beyond atrophy [14], while two radical prostatectomy studies demonstrated apoptosis and atrophy: [15] the effects of finasteride were similar but less pronounced than those of leuprolide and flutamide [16].

A prospective, randomized pilot study in men with prostate cancer undergoing radical prostatectomy has also examined the histological effects of the dual 5α-reductase inhibitor dutasteride. This study demonstrated significantly increased atrophy and decreased tumor volume, trends towards increased apoptosis and a higher treatment alteration score, and decreased microvessel density, for men treated for 6–10 weeks with 5 mg daily dutasteride versus placebo [17,18]. The objective of the current study was to further explore these findings by assessing the effect of 4 months therapy with dutasteride before radical prostatectomy compared with surgery alone on histopathological assessments of prostatic tissue in men with biopsy-proven, clinically localized prostate cancer.

MATERIALS AND METHODS

Study Population

Eligible men for this study were aged ≥45 and ≤80 years with a serum PSA of 2.5–10 ng/ml and biopsy-proven, localized prostate cancer (clinical stage T1c–T2b, N0/NX, M0) with a Gleason score ≤7. Those who had received prior treatment for prostate cancer were excluded. Other principal exclusion criteria included the use of 5α-reductase inhibitors or agents with androgenic or anti-androgenic properties within the last 12 months, recent use of selenium (>75 μg), vitamin E (>100 IU) or Saw Palmetto (a washout period of 2 weeks was acceptable for the latter two), or prior prostatic surgery (including minimally invasive techniques).

Study Design

This was a randomized, parallel-group study (Fig. 1). Prior to randomization, baseline assessments were conducted including examination of prostate biopsy cores to confirm Gleason score (for comparison with prostatectomy specimens), medical history, physical examination, and free and total serum PSA levels. Following a screening visit, subjects were randomized to one of three treatment arms in a 1:1:1 ratio: 0.5 mg dutasteride once daily for 4 months after a loading dose of 7 mg (to ensure that steady-state was achieved more rapidly), 3.5 mg dutasteride once daily for 4 months, or surgery alone at the earliest convenient time. The 0.5 mg dose is the approved dose for the treatment of benign prostatic hyperplasia, and is also being examined in the REDUCE prostate cancer prevention study [19]. The 3.5 mg dose was chosen to evaluate whether a larger dose of dutasteride would have more pronounced anti-tumor effects.

For subjects receiving dutasteride, the subjects and investigators were blinded as to dutasteride dose. For those randomized to surgery without dutasteride therapy, patients and investigators were aware of treatment allocation. Subjects randomized to dutasteride were required to return to the clinic for clinical assessments at 2 weeks (Visit 3), 2 months (Visit 4), and 4 months (Visit 5) after randomization. The 4-month post-randomization visit (Visit 5) occurred
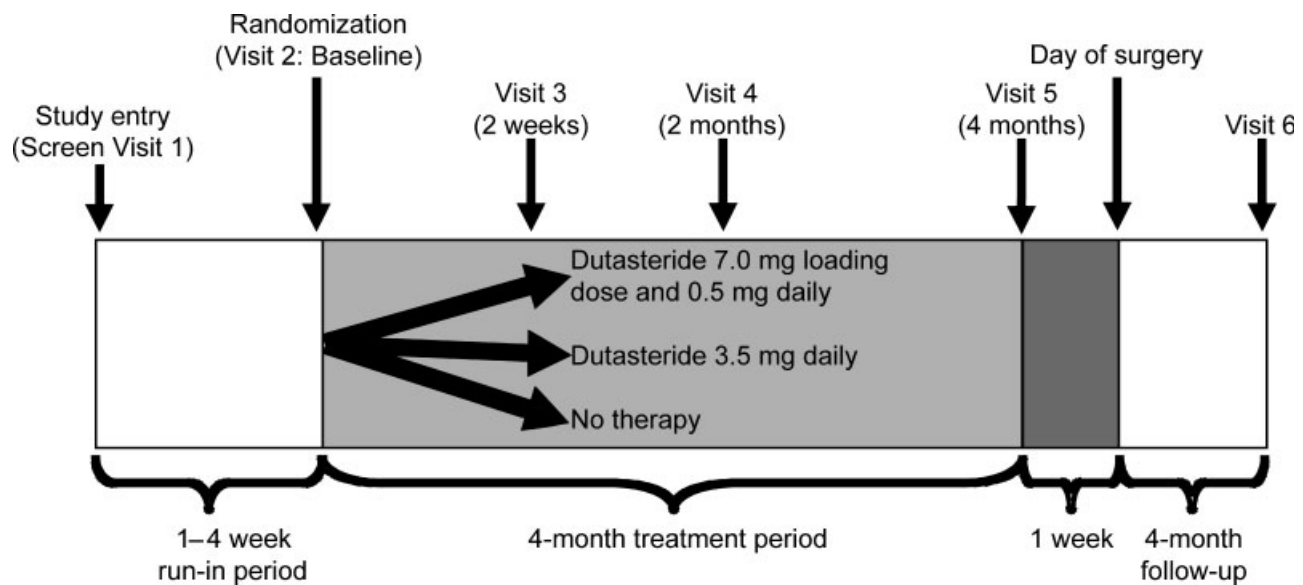
Fig. I. Summary of study protocol.

within 1 week before radical prostatectomy. At surgery, prostatectomy samples were obtained for histological analysis. All subjects returned for a follow-up visit 4 months after prostatectomy (Visit 6). All laboratory and histological assessments were conducted in a blinded fashion.

Serum and Intraprostatic Androgen and PSA Measurements

Serum levels of DHT, testosterone, and PSA were measured at Visits 2, 4, 5, and 6. Intraprostatic DHT and testosterone were measured in the benign portion of the resected prostate tissue. Androgens were measured by a highly sensitive gas chromatography/mass spectroscopy assay (PPD Development, Richmond, Virginia).

Prostate Volume Measurement by Ultrasound

Prostate volume measurements were conducted up to 3 months before screening or at baseline/randomization (Visit 2) and 4 months after randomization but before surgery (Visit 5). The anteroposterior, cephalocaudal, and transverse diameters of the prostate were obtained by TRUS/CDUS to calculate the prostate volume.

Apoptosis/Proliferation Markers and Morphological Parameters From Prostatectomy Specimens

Histopathological tissue samples were processed and the histology evaluated in a central pathology laboratory (Bostwick Laboratories, Richmond, Virginia). For the primary efficacy endpoint, tissue samples were evaluated for the percentage of cancer cell area

undergoing apoptosis as assessed by tissue transglutaminase (tTG) staining [20], which was conducted using unstained slides cut from blocks of prostate tissue taken on the day of surgery. In addition to tTG staining, TUNEL staining (percentage of prostatic cells per unit area undergoing apoptosis) was also conducted, and further assessments of proliferation, atrophy, microvessel density, tumor grade (Gleason score), nuclear and architectural changes (none, mild, moderate, or severe), stromal/epithelial ratio, and prostate cancer lesion number and size were also performed on all prostatectomy specimens. These are summarized in Table I.

Safety Assessments

Safety and tolerability assessments included physical examinations, vital signs, 12-lead ECG measurements (baseline only), clinical laboratory tests, and monitoring for adverse events.

Study Endpoints, Sample Size, and Study Power

The primary endpoint was the percentage of prostate cancer epithelial cell area undergoing apoptosis as assessed by tTG staining. Secondary endpoints included the number of benign and malignant prostatic epithelial cells per unit area undergoing apoptosis as assessed by TUNEL staining, the number of prostatic epithelial cells per unit area undergoing proliferation as assessed by Ki-67 labeling, microvessel density as assessed by CD34 staining, tumor grade (Gleason score), nuclear and architectural changes as assessed by the treatment alteration score, percentage of atrophic epithelium and the stromal/epithelial ratio.

TABLE I. Further Assessments of Prostatectomy Samples

Percentage of prostatic cells per unit area undergoing apoptosis as assessed by TUNEL staining
  Three randomly chosen microscopic fields containing at least 250 cells of each kind (stroma and epithelium in benign and cancer tissue) were evaluated
Percentage of prostatic cells per unit area undergoing proliferation as assessed by Ki-67 labeling
  Three 200× microscopic fields (0.754 mm^2) with maximum positive cells of each kind (stroma and epithelium in benign and cancer tissue) were evaluated
Microvessel density as assessed by CD34 staining
  Within the area of maximal CD34 expression, microvessels were counted on a 200× microscopic field (0.754 mm^2) for three separate fields. The average microvessel count (density) was reported for benign and cancer tissue separately
Tumor grade (Gleason score)
  The total Gleason score from the pre-study biopsy and at prostatectomy was documented
  The change in total Gleason score from pre-study to surgery was grouped into three categories: decrease in score, no change in score, or increase in score
Nuclear and architectural changes at prostatectomy as assessed by the treatment alteration score
  The treatment alteration score was an assessment of cytological changes characteristic of androgen deprivation: the sum of the nuclear treatment alteration score and the architectural treatment alteration score, each ranging from 0 to 3
Prostate cancer lesions
  Number and size of lesions
Percentage atrophic epithelium
  Percentage atrophic epithelium from benign tissue from the transitional and peripheral zone, HG-PIN, and cancer tissue at prostatectomy was evaluated at 10% increments
Stromal/epithelial ratio
  Stromal/epithelial ratio from benign, HG-PIN, and cancer tissue at prostatectomy was evaluated by image analysis for different regions of the specimen

HG-PIN = high-grade intraepithelial neoplasia.

Enrolment of approximately 26 randomized subjects per treatment group provided ≥90% power to declare superiority of the 3.5 mg dutasteride treatment versus surgery alone for the percentage of prostatic cell area undergoing apoptosis as assessed by tTG staining. This power estimate was based on the use of a two-sided *t*-test at the 0.05 significance level assuming 20 evaluable subjects per treatment group (assuming 23% randomized subjects non-evaluable), a mean of 1.2, and a standard deviation of 2.3 for the surgery alone group, and a mean of 4.5 for the 3.5 mg dutasteride group. These assumed values were based on the results of an earlier neoadjuvant study with dutasteride [17].

Analysis Populations and Statistical Methods

All efficacy analyses, including that of the primary endpoint, were based on the modified intention-to-treat (ITT) population, which consisted of all randomized subjects except those with no surgical tissue evaluation available. Serum DHT analyses were however conducted on the ITT population, which consisted of all randomized subjects. All values provided are means ± standard deviations unless otherwise stated. There were two comparisons of interest for the primary and secondary endpoint analysis: 3.5 mg dutasteride versus surgery alone and 0.5 mg dutasteride versus

surgery alone. For each comparison, two-sided tests of the null hypothesis were conducted at a significance level of 0.05. Treatment groups were compared using the log-rank test in the analysis of the primary endpoint.

RESULTS

Subject Demographics and Disposition

A summary of patient demographics is presented in Table II. Baseline characteristics were comparable between treatment groups. Mean total serum PSA was 6.2 ng/ml (range 2.6–18.35 ng/ml). Several subjects had a baseline PSA greater than 10 ng/ml; the majority of these had a rise in PSA between the screening and baseline visits. Median Gleason score was 6 (range 6–8), with 61% having a total score of <7, 37% having a score of 7, and one patient with a score of 8. This later patient was included in analyses, as the final Gleason score of 8 was assigned by a central pathologist after the initial assessment of the local pathologist.

A total of 81 subjects were randomized to treatment, with 75 completing the study. Subject accountability is presented in Figure 2. A similar proportion of subjects completed the study in each of the three treatment groups. The modified ITT population consisted of

TABLE II. Baseline Subject Characteristics for the Modified ITT Population (Mean ± Standard Deviation Unless Otherwise Specified)

Characteristic	Surgery alone (n = 25)	0.5 mg dutasteride (n = 26)	3.5 mg dutasteride (n = 24)
Age (years)	61.0 ± 5.71	60.0 ± 6.69	61.3 ± 5.35
Race (Caucasian)	92%	92%	92%
Total PSA (ng/ml)	6.3 ± 2.85	5.6 ± 2.08	6.7 ± 3.24
Prostate volume (cc)	37.0 ± 22.97	44.6 ± 23.84	40.9 ± 18.59
Total Gleason score at diagnosis (median)	6	6	6
Total Gleason score at diagnosis (mean)	6.37 ± 0.496	6.33 ± 0.483	6.53 ± 0.612
Gleason score <7	63%	67%	53%
Gleason score 7	37%	33%	42%
Gleason score 8–10	—	—	5%

75 men; 25 in the surgery-alone group, and 26 and 24 in the 0.5 and 3.5 mg dutasteride groups, respectively.

Serum and Intraprostatic Androgens

Mean changes in serum DHT from baseline for the three treatment groups are shown in Figure 3A. Treatment with dutasteride 0.5 mg resulted in pre-surgery suppression of DHT of −89.7 ± 6.07%, with a figure of −92.3 ± 4.4% for the 3.5 mg dose (both $P < 0.001$ versus the surgery-alone group). There was no change in the surgery-alone group. With the 3.5 mg dose of dutasteride, return towards pre-drug levels of DHT was less complete 4 months after therapy versus the 0.5 mg dose (−70.2% versus −25.7%).
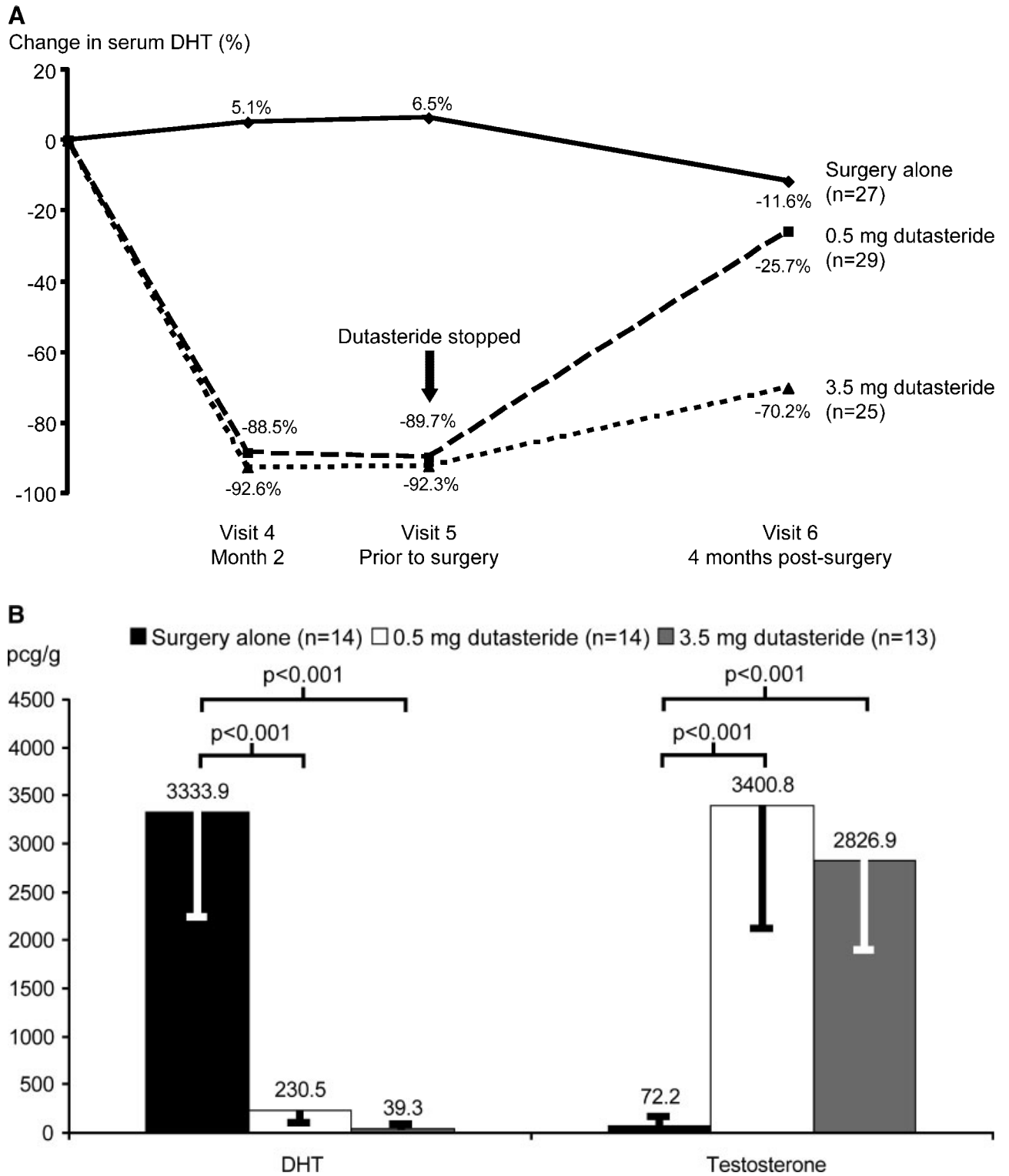
Mean serum testosterone concentrations rose from baseline to Visit 5 in subjects treated with dutasteride 0.5 and 3.5 mg by 16.1 ± 20.2% and 21.3 ± 21.18%, respectively, compared with an increase in the surgery-alone group of 4.6 ± 26.71% ($P = 0.026$ for 0.5 mg

dutasteride versus surgery alone; $P = 0.006$ for 3.5 mg dutasteride versus surgery alone). Four months following surgery, mean serum testosterone was similar to baseline in the surgery-alone group (0.3 ± 25.39% versus baseline), while levels remained above baseline in both dutasteride-treated groups (9.6 ± 25.51% and 15.5 ± 27.42% for the 0.5 and 3.5 mg dose groups, respectively).

Two subjects, one in the surgery-alone group and the other in the 0.5 mg dutasteride group, had intraprostatic DHT data that were inconsistent with their treatment allocation. Data are therefore presented for the ITT population without these two outliers. As both samples came from the same center on the same day, the most likely explanation is an inadvertent switch of the samples. Mean intraprostatic DHT levels were significantly lower in subjects who received dutasteride 0.5 or 3.5 mg versus the surgery-alone group (Fig. 3B). This represented 93.1% and 98.8% lower mean DHT for subjects receiving dutasteride 0.5 and 3.5 mg,
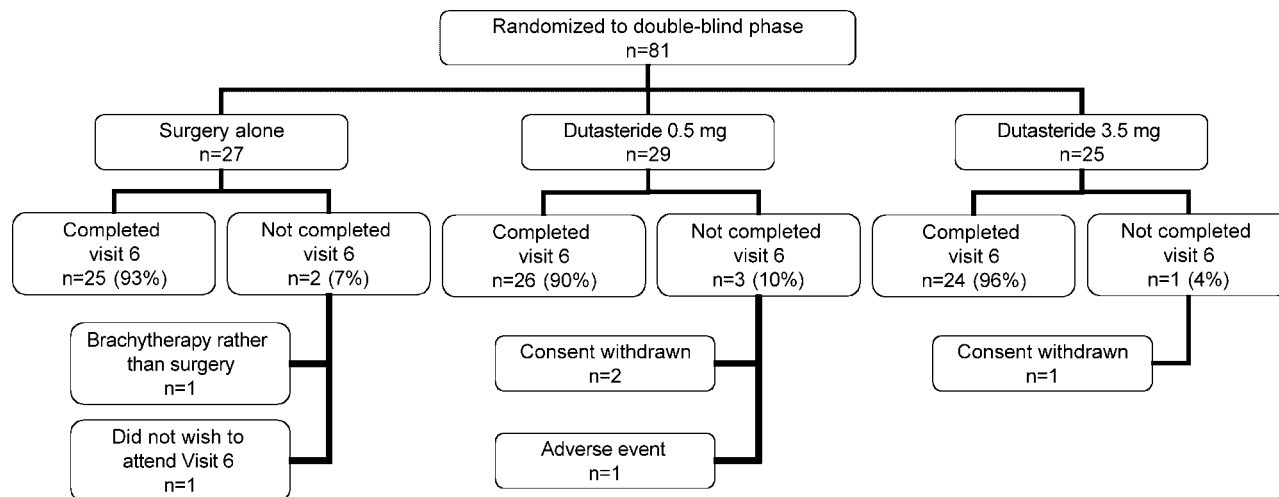
Fig. 2. Subject accountability.

A
Change in serum DHT (%)

B

Fig. 3. **A**: Mean percentage change in serum DHT concentration from baseline by treatment group. **B**: Mean ($\pm$ standard deviation) intraprostatic DHT and testosterone concentrations (excluding two outliers) measured following prostatectomy, by treatment group.

respectively, versus surgery alone. Intraprostatic testosterone levels were significantly higher in subjects who received dutasteride 0.5 or 3.5 mg versus the surgery-alone group (Fig. 3B).

Serum PSA and Prostate Volume

Serum PSA changed little in the surgery-alone group prior to surgery, with a decrease from baseline of

5.9 ± 19.16% at Visit 5. In contrast, treatment with dutasteride 0.5 and 3.5 mg resulted in mean decreases of 47.1 ± 19.24% and 58.0 ± 17.92%, respectively, over the same period, which were statistically significant compared with surgery alone ($P < 0.001$ for both dutasteride groups). Following surgery, serum PSA decreased in all three groups from baseline by 98.5 ± 1.55%, 98.9 ± 0.44%, and 99.0 ± 0.43% for the surgery-alone, dutasteride 0.5 and 3.5 mg groups, respectively. From baseline to the final assessment prior to surgery, prostate volume rose by 1.8 ± 20.72% in the surgery-alone group versus decreases of 16.6 ± 19.33% and 19.7 ± 19.59% for the dutasteride 0.5 mg ($P = 0.020$) and 3.5 mg ($P = 0.002$) groups, respectively.

Morphological Parameters and Apoptosis/Proliferation Markers

Benign tissue. A comparison of morphological parameters and apoptosis/proliferation markers of benign prostatic tissue by treatment group is shown in Table III. Treatment with dutasteride was associated with a greater proportion of atrophic epithelium in both the peripheral and transition zones versus surgery alone, but this only reached statistical significance for the 0.5 mg dutasteride group. The stromal/epithelial ratio was similar between treatment groups, while there was a trend to increased microvessel density in the dutasteride groups versus the surgery-alone group. There was no significant effect of dutasteride on apoptosis, and epithelial, but not stromal proliferation was increased versus surgery alone.

Prostate cancer tissue. A comparison of morphological parameters and apoptosis/proliferation markers of prostate cancer tissue by treatment group is shown in

Table IV. As no prostatectomy specimen had more than two lesions within it, the volume of the largest and second largest cancers were summated to provide total tumor volume. One subject in each dutasteride group had a tumor volume 4.5 standard deviations above the mean (tumor volume 17 cc in the 0.5 mg group and 38 cc in the 3.5 mg group). When these two outliers were removed, the mean tumor volumes were 1.37 and 1.70 cc for the 0.5 mg group and 3.5 mg groups, respectively. The differences between the two dutasteride groups with the outliers removed and the surgery-alone group were statistically significant ($P = 0.02$ for the 0.5 mg group and $P = 0.03$ for the 3.5 mg group), as was the difference for all dutasteride subjects combined versus surgery alone ($P = 0.01$).

The proportion of atrophic epithelium was lower, and treatment alteration scores were greater, in dutasteride-treated subjects versus the surgery-alone group, but these differences were not statistically significant. As with the benign tissue samples, microvessel density was elevated in dutasteride-treated subjects versus the surgery-alone group. The proportions of tumor cells classified as apoptotic, and differences between the treatment groups, were not consistent between the two methods of assessment (tTG and TUNEL). For tTG, the primary endpoint, staining demonstrated a trend to increased apoptosis in dutasteride-treated subjects versus surgery alone, while the TUNEL staining demonstrated a significant decrease in apoptosis in dutasteride-treated subjects versus surgery alone. Proliferation was increased in dutasteride-treated subjects versus surgery alone, although this was only statistically significant for the 0.5 mg dose.

The mean Gleason score increased from biopsy to prostatectomy in each of the three treatment groups, with the median rising from 6 to 7 in each case. Changes

TABLE III. Comparison of Histological Parameters and Epithelial Apoptosis/Proliferation Markers of Benign Prostatic Tissue by Treatment Group (Mean ± Standard Deviation; P-Values versus Surgery Alone)

Characteristic	Surgery alone (n = 25)	0.5 mg dutasteride (n = 26)	3.5 mg dutasteride (n = 24)
Morphology			
Peripheral zone atrophic epithelium	26.8 ± 21.74%	40.4 ± 23.91%, $P = 0.026$	36.7 ± 26.81%, $P = 0.16$
Transitional zone atrophic epithelium	15.2 ± 9.63%	21.2 ± 9.09%, $P = 0.018$	16.7 ± 10.50%, $P = 0.61$
Stromal cells	59.6 ± 7.41%	57.2 ± 12.35%, $P = 0.65$	59.7 ± 7.35%, $P = 0.90$
Microvessel density (vessels per mm^2)	57.0 ± 19.86	62.5 ± 15.10, $P = 0.52$	66.9 ± 23.12, $P = 0.18$
Apoptosis and proliferation			
Apoptotic cells by TUNEL staining			
Stroma	0.22 ± 0.31%	0.22 ± 0.29%, $P = 0.77$	0.26 ± 0.44%, $P = 0.76$
Epithelium	0.99 ± 2.40%	0.53 ± 0.50%, $P = 0.62$	0.51 ± 0.32%, $P = 0.49$
Proliferating cells by Ki-67 labeling			
Stroma	0.53 ± 0.52%	0.56 ± 0.43%, $P = 0.97$	0.73 ± 0.64%, $P = 0.40$
Epithelium	1.09 ± 0.74%	1.74 ± 1.13%, $P = 0.022$	1.68 ± 0.89%, $P = 0.009$

TABLE IV. Comparison of Histological Parameters and Epithelial Apoptosis/Proliferation Markers of Prostate Cancer Tissue by Treatment Group (Mean ± Standard Deviation; *P*-Values Versus Surgery Alone)

Characteristic	Surgery alone (n = 25)	0.5 mg dutasteride (n = 26)	3.5 mg dutasteride (n = 24)
Morphology			
Total tumor volume			
Median (cc)	2.03	1.19	1.13
Mean (cc)	2.30	1.97	3.22
Atrophic epithelium	13.2 ± 24.79%	8.1 ± 17.67%, $P = 0.87$	10.4 ± 16.81%, $P = 0.38$
Stromal cells	26.0 ± 8.82%	28.6 ± 12.34%, $P = 0.42$	29.9 ± 11.48%, $P = 0.18$
Microvessel density (vessels per mm^2)	71.4 ± 27.27	90.3 ± 34.14, $P = 0.031$	83.3 ± 23.67, $P = 0.26$
Treatment alteration score	0.64 ± 1.25	0.85 ± 1.38, $P = 0.57$	0.79 ± 1.50, $P = 0.70$
Nuclear treatment alteration score	0.36 ± 0.64	0.42 ± 0.76, $P = 0.75$	0.33 ± 0.64, $P = 0.88$
Architectural treatment alteration score	0.28 ± 0.68	0.42 ± 0.70, $P = 0.46$	0.46 ± 0.88, $P = 0.43$
Apoptosis and proliferation			
Cancer area staining positive for tTG	0.30 ± 0.85%	1.17 ± 2.30%, $P = 0.21$	1.01 ± 2.52%, $P = 0.24$
Apoptotic epithelial cells by TUNEL staining	2.50 ± 3.07%	1.30 ± 1.75%, $P = 0.025$	1.24 ± 1.08%, $P = 0.046$
Proliferating epithelial cells by Ki-67 labeling	4.93 ± 3.14%	7.63 ± 6.10%, $P = 0.038$	5.32 ± 3.82%, $P = 0.70$

in score from baseline to prostatectomy are summarized in Figure 4. Gleason scores were more frequently elevated between biopsy and prostatectomy in the surgery-alone group than in either of the dutasteride groups.

Safety

Subjects in the dutasteride groups waited twice as long before surgery compared with the surgery-alone group (126 days versus 49.5 days). Seventeen adverse events in 12 (22%) subjects were considered by the investigators to be related to study drug, none of which were serious. Fifteen drug-related events with an onset during treatment occurred in ten (19%) subjects and two drug-related events with an onset post-treatment occurred in two (4%) subjects. The drug-related adverse events with an onset during treatment were decreased libido, loss of libido, erectile dysfunction, ejaculation failure, perineal pain, nausea, abdominal distension, fatigue, decreased semen volume, dizziness, and headache. There were no clinically important differences among dutasteride treatment groups in the incidence of drug-related events. There were no
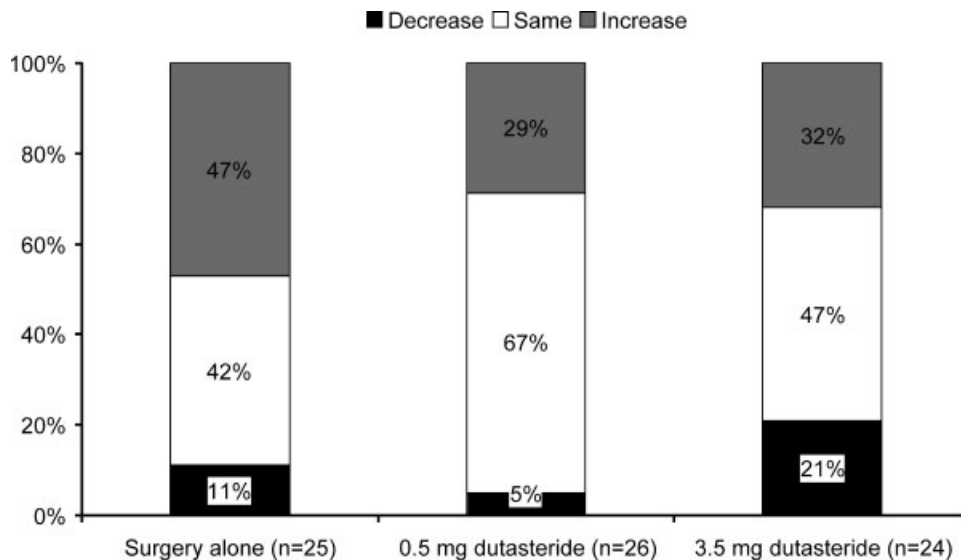
Fig. 4. Percentage of prostate cancers with a decreased, same or increased Gleason score between biopsy and radical prostatectomy.

drug-related adverse events in the surgery-alone group. Adverse events after surgery were comparable among the groups. There were no clinically important differences among treatment groups in any measures of cardiovascular function, hematology, or clinical chemistry parameters.

DISCUSSION

The results of this study confirm those of previous studies, which found that treatment with dutasteride at doses of ≥ 0.5 mg daily results in suppression of both serum [17,21,22] and intraprostatic DHT levels [17] to a near-maximal $\geq 90\%$ of baseline values. This study also confirms the effects of dutasteride in reducing prostate volume and serum PSA within a few months of treatment [22]. The magnitude of the reductions in serum PSA (47.1% with 0.5 mg and 58.0% with 3.5 mg) is similar to that seen in men with benign prostatic hyperplasia treated with dutasteride 0.5 mg for 6 months or longer [23]. This suggests that either the tumor tissue in the present study contributed little to serum PSA, or that dutasteride suppresses PSA production from both benign and malignant prostate tissue. The fact that tumor volumes were lower in the dutasteride groups of the present study is consistent with an effect of dutasteride on tumor tissue itself. This observation could raise concern that 5α-reductase inhibitors might decrease the utility of PSA for the diagnosis of prostate cancer. However, recent evidence from the PCPT demonstrates that treatment with a 5α-reductase inhibitor enhances detection of significant prostate cancer by increasing the area under the receiver operator characteristic curve for PSA [24]. One hypothesis to explain this phenomenon is that by reliably suppressing the benign component of PSA secretion, 5α-reductase inhibitors increase the ability of PSA increases over time to reflect growth of clinically meaningful prostate cancer.

If dutasteride reduces prostate tumor volume, it would seem intuitive that it should also affect biomarkers of androgen action. Previous data for the effects of 5α-reductase inhibitors on prostate cancer morphology are limited, but demonstrate that treatment with finasteride results in changes such as apoptosis and pyknosis, small tumor glands and lymphocytic infiltration to a lesser degree than that seen with androgen ablation [16]. The effects of neoadjuvant androgen ablation for 3 months in decreasing tumor volume with underlying tumor epithelial atrophy are also well known [25]. In the current study, pre-surgical treatment with dutasteride resulted in lower tumor volumes, without significant changes in treatment alteration scores or atrophy versus those randomized to surgery-alone. A previous study has demonstrated a significant decrease in the percentage of specimen involved with cancer, a significantly higher percentage of atrophic epithelium, and a trend towards an increased treatment alteration score with pre-surgical dutasteride versus placebo [18]. These data lend support to the hypothesis that dutasteride therapy results in similar, but lesser, changes compared with androgen ablation.

With regard to tumor cell apoptosis and proliferation, one study examining TUNEL staining [26], and a further study using histological assessment known to highly correlate with TUNEL [27], have noted that apoptosis is evident in the few days following initiation of androgen ablation therapy, with staining returning to baseline thereafter [26,27]. This finding is supported by the observation that levels of apoptosis in prostate cancer tissue correlate with duration of therapy over the course of 3–7 months, but not over 8–12 months, again demonstrating that apoptosis occurs early during androgen ablation [28]. Previous data on tTG staining have demonstrated that tissue expression is lower in prostate cancer versus normal or hyperplastic glands [29], lower with higher tumor grade, substantially down-regulated in metastatic disease [30], and tends to be higher in men receiving neoadjuvant treatment than in those with untreated cancer [20], with a longer duration of staining than TUNEL [31,32]. The same time-dependent effect has also been noted for Ki-67 staining for cellular proliferation. Decreased proliferation occurs in the few days following initiation of neoadjuvant therapy, but proliferation increases to greater than baseline levels thereafter [26,27]. It can be hypothesized that androgen-sensitive cells undergo apoptosis early during treatment, and that the subsequent decline in apoptosis represents the selective survival of relatively androgen ablation-resistant tumor cells.

It is evident from these studies that the timing of assessment for apoptosis and proliferation is critical. Within a few days to weeks, apoptosis visualized with TUNEL staining is prominent, with tTG staining persisting for longer. Tumor cell proliferation is initially decreased, but increases beyond this early phase of therapy. The data for dutasteride appear to follow this pattern. In a recent study in which treatment with dutasteride was administered for 5–11 weeks prior to radical prostatectomy, there were trends towards decreased proliferation and increased apoptosis (by TUNEL and tTG staining) in prostate cancer specimens with dutasteride versus placebo [17]. In the present study, where therapy was continued to 4 months, there was a non-significant elevation in tTG staining, a significant decrease in TUNEL staining, and evidence of an increase in proliferation with dutasteride versus surgery alone.

Microvessel density in the prostate assessed by CD34 staining is elevated in prostate cancer, and has been shown to correlate with tumor grade [33]. It might seem intuitive therefore that neoadjuvant 5α-reductase inhibitor therapy should be associated with a significant decrease in microvessel density. In benign prostatic tissue, treatment with finasteride has been shown to reduce microvessel density in both humans [34–36] and rats; [37] an observation that has been proposed to explain a reduction in hemorrhage seen with pre-operative finasteride treatment in men undergoing transurethral resection of the prostate [34]. However, data for the effects of neoadjuvant 5α-reductase inhibitor therapy in men with prostate cancer are lacking. Data are available for microvessel density from just one published study comparing men who received androgen ablation or no therapy prior to surgery. There was a minor, non-significant elevation in microvessel density in men who had received therapy versus those who had not [38]. In the present study, a minor elevation in microvessel density was also observed. From present data with androgen ablation and 5α-reductase inhibitor therapy, it is possible that androgen deprivation has a neutral effect on tumor microvasculature, while decreasing benign prostate volume and tumor size [39]. The result would be an increase in microvessel density despite a neutral effect on microvessel number.

In the PCPT, a significant reduction in the 7-year period prevalence of prostate cancer was observed for men who received daily finasteride therapy versus placebo [12]. However, finasteride treatment was associated with an excess risk of a high-grade tumor diagnosis, prompting concerns that it may selectively promote aggressive tumors [40]. There is now evidence that this reflects an enhanced detection rate, through the known effect of 5α-reductase inhibitors in reducing prostate volume, rather than the induction or selection of high-grade disease [41]. In support of this hypothesis, Gleason scores were more frequently elevated between biopsy and prostatectomy in the surgery-alone group than in either of the dutasteride groups in this study, demonstrating that dutasteride did not enhance the growth of higher-grade tumors over this treatment period.

CONCLUSIONS

Pre-surgical treatment with dutasteride in men with localized prostate cancer was associated with reductions in serum and intraprostatic DHT of $\geq 90\%$, which resulted in a significant decrease in overall prostate volumes and a numerical decrease in tumor volumes. No effect of dutasteride was noted on Gleason grade. With regard to apoptosis and proliferation, it is likely

that after 4 months of therapy with dutasteride, it is too late to see the early decrease in proliferation and increase in apoptosis (measured by TUNEL staining) that have been observed in studies using androgen ablation. Microvessel density alterations are similar to the limited data for androgen ablation, suggesting that tumor microvessel number is unaffected by dutasteride therapy, but that decreases in prostate and tumor volume do occur, resulting in increased microvessel density. Overall therefore, as with an earlier, small-scale study with finasteride showing that tumor effects were similar but less prominent than those seen with leuprolide and flutamide [16], it appears that dutasteride treatment results in similar but less marked changes compared with androgen ablation. Ongoing studies are evaluating the effects of dutasteride on other relevant biomarkers of treatment stress, androgen receptor activity, and signal transduction. Ultimately, the role of 5α-reductase inhibitors in the treatment of prostate cancer remains to be defined, although evidence for their effects on benign epithelium is probably also relevant to their now demonstrated role in the chemoprevention of prostate cancer [12].

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