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ORIGINAL REPORT

Targeted Androgen Pathway Suppression in Localized Prostate Cancer: A Pilot Study

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Ligand-mediated activation of the androgen receptor (AR) is critical for prostate cancer (PCa) survival and proliferation. The failure to completely ablate tissue androgens may limit suppression of PCa growth. We evaluated combinations of CYP17A and 5-α-reductase inhibitors for reducing prostate androgen levels, AR signaling, and PCa volumes.

Patients and Methods
Thirty-five men with intermediate/high-risk clinically localized PCa were randomly assigned to goserelin combined with dutasteride (ZD), bicalutamide and dutasteride (ZBD), or bicalutamide, dutasteride, and ketoconazole (ZBDK) for 3 months before prostatectomy. Controls included patients receiving combined androgen blockade with luteinizing hormone-releasing hormone agonist and bicalutamide. The primary outcome measure was tissue dihydrotestosterone (DHT) concentration.

Results
Prostate DHT levels were substantially lower in all experimental arms (0.02 to 0.04 ng/g *v* 0.92 ng/g in controls; $P < .001$). The ZBDK group demonstrated the greatest percentage decline in serum testosterone, androsterone, and dehydroepiandrosterone sulfate ($P < .05$ for all). Staining for AR and the androgen-regulated genes prostate-specific antigen and TMPRSS2 was strongly suppressed in benign glands and moderately in malignant glands ($P < .05$ for all). Two patients had pathologic complete response, and nine had ≤ 0.2 cm^3 of residual tumor (defined as a near-complete response), with the largest numbers of complete and near-complete responses in the ZBDK group.

Conclusion
Addition of androgen synthesis inhibitors lowers prostate androgens below that achieved with standard therapy, but significant AR signaling remains. Tissue-based analysis of steroids and AR signaling is critical to informing the search for optimal local and systemic control of high-risk prostate cancer.

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INTRODUCTION

Therapeutic approaches that reduce circulating testosterone are the most effective treatments available for metastatic prostate cancer. Strategies designed to impede androgen receptor (AR) signaling have also shown beneficial effects in preventing or treating localized prostate cancers.[1,2] However, the efficacy of androgen deprivation therapy (ADT) is limited by the inability to consistently reduce tissue androgens to below levels that activate AR signaling. Despite reduction of serum testosterone and dihydrotestosterone (DHT) levels to lower limits of assay quantification, prostate androgens in patients who have undergone castration remain at 25% to 35% of levels in untreated patients.[3-5] Consistent with these high residual androgen levels, persistent expression of AR-regulated genes is noted in prostate epithelium after ADT.[5,6] The inability to completely ablate androgens and AR signaling is reflected in the low number of complete clinical responses observed in studies of neoadjuvant ADT, with pathologic complete responses reported in only 4% of men treated with 3 to 8 months of ADT before prostatectomy.[7,8] These observations suggest the AR program continues to function as a key survival factor and that efforts culminating in AR program extinction may produce more substantial response rates.

This study was designed to determine whether combinations of agents targeting testicular, adrenal, and prostate androgen production would suppress prostate androgens and AR signaling more effectively than testicular androgen suppression alone, and, secondarily, whether this would enhance pathologic responses. We used the luteinizing hormone-releasing hormone (LHRH) agonist goserelin to reduce serum testosterone; the 5-α-reductase (SRD5A) inhibitor dutasteride to inhibit conversion of testosterone to the more potent androgen DHT, the CYP11A1/CYP17A1 inhibitor ketoconazole to block production of adrenal androgen precursors, and the AR antagonist bicalutamide to further inhibit AR signaling by remaining androgenic ligands. We used a neoadjuvant strategy to directly assess the efficacy of these agents on their targeted pathways and to permit quantitative measures of tissue androgens and residual tumor volumes.

PATIENTS AND METHODS

Patient Population

This was a randomized, unblinded, parallel-group study. All procedures were approved by institutional review boards of University of Washington, Veterans Affairs Puget Sound, and Dana-Farber Cancer Institute, and all subjects signed written informed consent. Eligible men had localized prostate cancer (T1c-T3, N0/NX,) with Gleason score ≥ 7. Patients with a risk of nodal involvement more than 10% were required to have negative bone scan and computed tomography of the abdomen/pelvis. Prior therapy for prostate cancer, including drugs affecting androgen or ketoconazole metabolism, history of thrombosis, unstable angina, or heart failure were exclusionary. Men were required to have a serum testosterone ≥ 280 ng/dL and normal blood counts, creatinine, and transaminases.

Study Procedures

Patients were randomly assigned to 3 months of neoadjuvant therapy with (1) goserelin (Zoladex; AstraZeneca, London, United Kingdom) 10.8 mg with high-dose dutasteride (Avodart, GlaxoSmithKline, London, United Kingdom) 3.5 mg per day (ZD; n = 12); (2) goserelin, bicalutamide (Casodex; AstraZeneca) 50 mg per day, and dutasteride (ZBD; n = 12); or (3) goserelin, bicalutamide, dutasteride, and ketoconazole 200 mg three times per day (with prednisone 5 mg per day; ZBDK; n = 13). Otherwise eligible men who presented to clinic already receiving combined androgen blockade with an LHRH agonist and bicalutamide underwent prostatectomy with tissue acquisition at 3 months as controls (ZB, n = 8). A group of untreated patients who met enrollment criteria were included for comparison (n = 11). All patients underwent open retropubic prostatectomy.

Tissue Acquisition and Determination of Tumor Volume

Transverse 2- to 4-mm-thick sections of the prostate were made from apex to base and divided into quadrants. Alternating cross-sections were submitted for formalin fixation or snap frozen. Hematoxylin and eosin–stained slides were used for pathologic staging (International Society of Urological Pathology guidelines)[9] and determination of tumor volume by tissue morphometry.

Androgen Measurements

Serum and tissue androgen levels were determined by mass spectrometry in a blinded manner, using a modification of methods we have previously described.[10] Benign prostate tissue was macro-dissected from snap-frozen prostatectomy tissue (owing to limitations and variability in the amount of residual tumor tissue present in the frozen tissue available for analysis). The lower limit of quantitation in serum was 0.49 pg/sample for testosterone, DHT, and androstenedione (AED) and 3.9 pg/sample for dehydroepiandrosterone (DHEA) and in tissue was 0.49 pg/sample for testosterone and 0.98 pg/sample for DHT, AED, and DHEA. Intra-assay coefficients of variation generated using human serum for high-, mid-, and low-range samples were 5.4%, 4.8%, and 5.2% for testosterone; 6.4%, 6.2%, and 17.2% for DHT; 4.6%, 4.2%, and 3.0% for AED; and 2.9%, 3.7%, and 5.5% for DHEA. Serum levels of DHEA sulfate (DHEA-S) were measured as free DHEA after sulfatase treatment. Free DHEA and androsterone were measured using a Girard testosterone derivatization strategy in conjunction with a stable isotope dilution liquid chromatography electrospray ionization selected reaction monitoring mass spectrometry method that we have recently established (Tamae et al, manuscript submitted for publication).

Immunohistochemistry

A prostate tissue microarray comprising six benign and cancer containing cores (0.6 mm) was created using fixed blocks obtained at prostatectomy. Serial sections were stained using an autostainer (Dako North America, Carpinteria, CA) with primary antibodies against AR (BioGenex antibody F39.4.1, 1:500; BioGenex, Fremont, CA); prostate-specific antigen (PSA) (Dako antibody A562, 1:50), and TMPRSS2 (previously described[11]). Immunostaining was assessed in a blinded manner using a compositional method for the percentage of cells at each intensity level (no stain, weak stain, intense stain). The mean percentage with high-intensity immunohistochemistry (IHC) expression was separately tabulated in benign and cancer glands for each subject. Data are displayed as stacked bar graphs representing the mean percentage staining at each intensity level in benign or malignant prostate epithelium for each treatment group.

Statistical Analyses

Linear regression models were used to compare prostate and serum androgens in each protocol treatment arm (ZD, ZBD, and ZBDK) to the standard treatment arm (ZB). Measurements were log-transformed to satisfy normality assumptions, Dunnett's adjustment was used to correct for multiplicity,[12] and a sandwich estimator of the covariance matrix was used to account for variance across treatment groups.[13] For IHC analyses, the mean percentage high staining in tissues from the control arm receiving combined blockade (ZB) and the grouped study arms (ZD, ZBD, and ZBDK) were compared with the mean percentage high-intensity staining in untreated tissues using Welch's *t* tests. Significant differences in intense staining for AR, PSA, and TMPRSS2 in benign or cancer tissue were not observed among the three study arms (ZD, ZBD, and ZBDK), except for lower percent intense PSA staining in benign tissue from the ZBD cohort compared with ZD and ZBDK, so data from these cohorts were grouped together.

Differences in serum androgens, tissue androgens, tumor volume, and prostate IHC score stratified by PSA nadir were evaluated by Welch's *t* tests. The PSA nadir was dichotomized at 0.2 based on previous reports demonstrating lower tumor volume and relapse rates in patients treated with ADT combined with surgery.[7,14,15] The dependence of time to PSA relapse on change in serum and tissue androgens, PSA nadir, tumor volume, and treatment group was evaluated using Cox proportional hazards models. The proportional hazards assumption was tested with the cox.zph function in the R survival package.

RESULTS

Patient Characteristics

This study enrolled 35 patients with intermediate to high-risk prostate adenocarcinoma (Fig 1; Table 1). Although patients were randomly assigned to the three treatment arms, they were not stratified, and a higher proportion of patients with intermediate-risk prostate cancer were treated in the ZBDK cohort, compromising conclusions about clinical efficacy. All patients had PSA decline, and there was no evidence of disease progression during treatment.

Prostate Tissue Androgen Levels

The primary end point was whether addition of SRD5A and CYP17A inhibition would suppress tissue DHT levels below that achieved with combined androgen blockade using an LHRH agonist

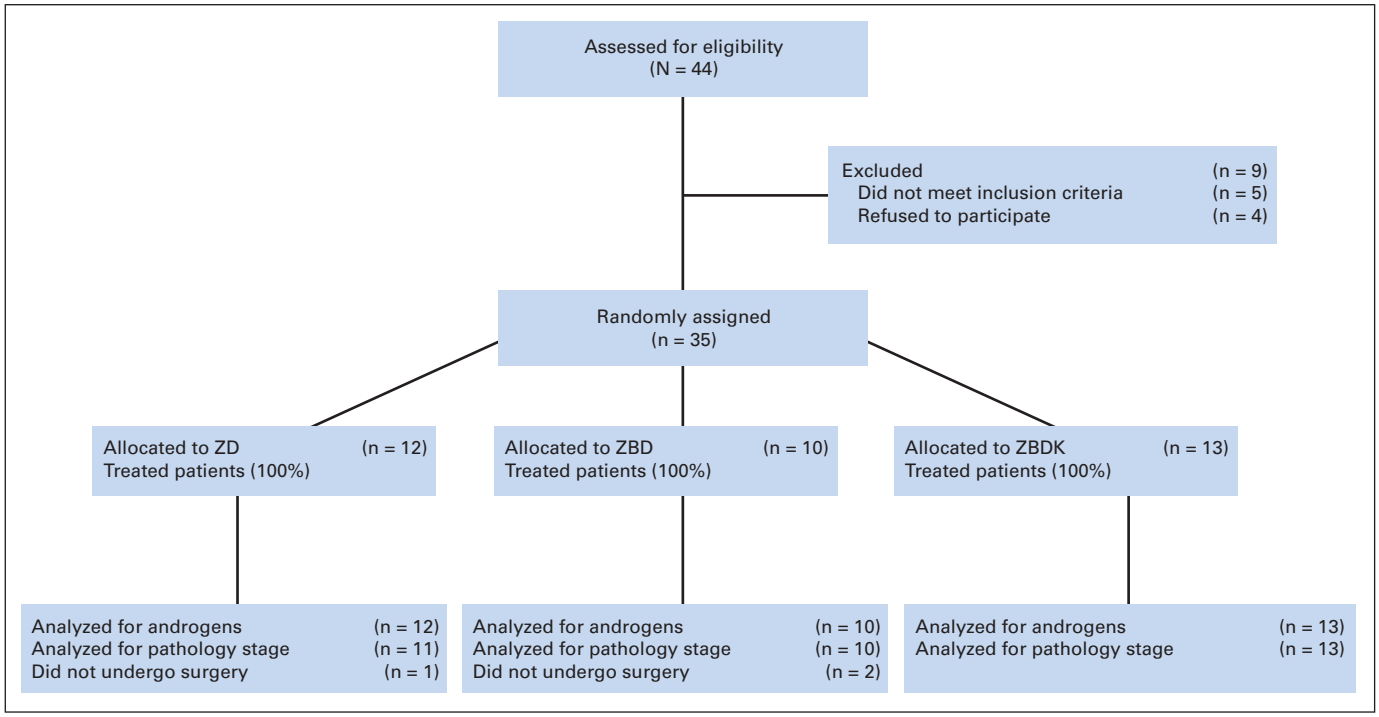

Fig 1. CONSORT diagram. ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.

and bicalutamide (ZB group). All experimental cohorts achieved the study end point, with DHT levels of ~0.03 to 0.06 ng/g (0.11 to 0.19 nmol/L) in the ZD, ZBD, and ZBDK groups compared with 0.92 ng/g (3.2 nmol/L) in the control ZB group ($P < .05$ for all; Fig 2A and Table 2).

Consistent with the effect of SRD5A inhibition, prostate tissue testosterone levels in all dutasteride-containing cohorts (0.24 to 0.33 ng/g; 0.8 to 1.1 nmol/L) were higher than those in men treated with combined blockade alone (0.07 ng/g; 0.25 nmol/L, Fig 2B and Table 2), even in the cohort (ZBDK) receiving the CYP17A inhibitor ketoconazole. Levels were numerically but not statistically higher than those in untreated patients (0.26 ng/g) and were an order of magnitude lower than those previously reported in eugonadal men treated with 3.5 mg of dutasteride.[16] Similar to the changes observed for testosterone, AED levels were significantly higher in tissues from dutasteride-treated ZD and ZBD patients (1.94 to 2.58 ng/g *v* 0.70 ng/g in ZB, Fig 2C and Table 2). The increase in AED in the ketoconazole containing arm (1.61 ng/g) trended toward significance ($P = .104$), suggesting that CYP17A inhibition attenuated AED synthesis. Tissue DHEA levels in the dutasteride containing arms were not statistically different from each other (Fig 2D, Table 2). The ZBD and ZBDK groups did show a trend toward a statistically significant decrease in prostate DHEA levels when compared with the standard combined blockade arm (ZB).

Table 1. Baseline Clinical Characteristics of Control and Study Patients

Characteristic	Untreated Patients (n = 11)	Control Patients (ZB, n = 8)*	Study Patients		
			ZD (n = 12)	ZBD (n = 10)†	ZBDK (n = 13)
Median age, years (range, 41-82 years)	59	61	62	66	60
Clinical staging and Gleason score					
cT1c	6	1	3	2	5
cT2a/b	4	5	5	6	7
cT2c/T3	1	1	4	2	1
7/8-10	8/1	1/5	6/6	9/3	12/1
PSA characteristics					
Median PSA	4.7	6.8	11.9	5.8	7.9
< 10/10-20/> 20	10/10	4/12	4/4/4	8/1/1	10/1/2

Abbreviations: PSA, prostate-specific antigen; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.
*Two patients in this group underwent end-of-study biopsy instead of prostatectomy (one withdrew, one had radiation therapy).
†One patient in this group underwent end-of-study biopsy instead of prostatectomy (because of diagnosis of lung cancer).

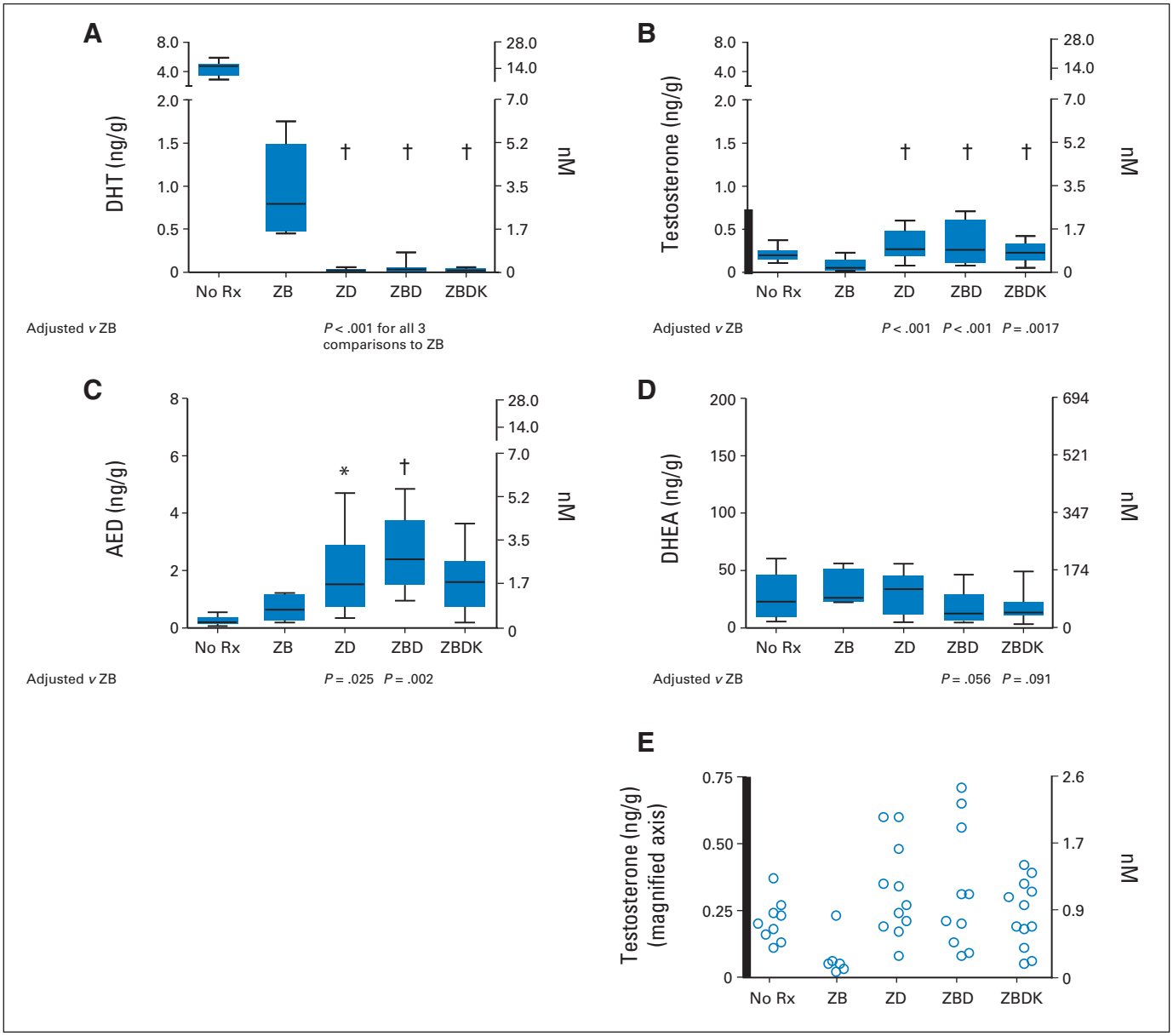

Fig 2. Prostate androgen levels after 3 months of multitargeted neoadjuvant androgen suppression. Tissue androgens from prostatectomy specimens after no treatment (No Rx), or 3 months of combined androgen blockade with a luteinizing hormone-releasing hormone agonist (goserelin) and bicalutamide (ZB); goserelin combined with the SRD5A inhibitor, dutasteride (ZD); goserelin combined with bicalutamide and dutasteride (ZBD); and all three of these agents combined with the CYP17A inhibitor, ketoconazole (ZBDK). Levels of (A) dehydrotestosterone (DHT), (B) testosterone, (C) androstenedione (AED), and (D) dehydroepiandrosterone (DHEA) were measured by mass spectroscopy. The difference in tissue androgen levels between each treatment cohort and the control (ZB) group was evaluated by linear regression. Statistically significant *P* values ($P < .05$) or those trending toward significance ($P < .10$) are presented in italics below the relevant treatment group. Absolute values of the mean and standard deviations are presented in Table 2. (E) Residual prostate testosterone levels are shown on an expanded *x*-axis (corresponding to the region denoted with a dark vertical bar in panel B). (*) $P < .05$. (†) $P < .005$.

To better approximate residual androgen activity in the prostate, we evaluated an androgen index designed to reflect the combined ligand activity of testosterone and DHT. Although both testosterone and DHT are high-affinity ligands for the AR, DHT has three- to five-fold higher potency relative to testosterone.[17] Therefore, we estimated the androgen index as the sum of (5 × DHT) + (1 × testosterone concentration) for each cohort (Table 2). Notably, the combined activity of residual testosterone and DHT levels in the prostate remain at concentrations capable of activating the AR, which has been reported to occur in vitro at DHT concentrations as low as 10 to 14 mol/L in the setting of prolonged androgen deprivation.[18]

Serum Androgen Levels

All treatment arms achieved castrate (< 50 ng/dL) levels of serum testosterone (range, 9.4 to 27.2 ng/dL; Appendix Table A1, online only). We evaluated the paired data from the pre and post-treatment samples in each cohort and computed percentage change as a more accurate indicator of treatment effect. The magnitude of decline in serum DHT levels was significantly higher in patients treated with

Table 2. Post-Treatment (RP) Prostate Tissue Androgen Levels

Treatment Category	DHT (ng/g)			Testosterone (ng/g)			AED (ng/g)		DHEA (ng/g)		Androgen Index* (nM)
	Mean	SD	nM	Mean	SD	nM	Mean	SD	Mean	SD	
No treatment	4.38	0.99	15.2	0.26	0.17	0.73	0.26	0.17	28.2	21.8	76
ZB	0.92	0.49	3.20	0.07	0.08	0.25	0.70	0.41	33.5	14.2	16.3
ZD	0.03	0.01	0.12	0.32	0.17	1.11	1.94	1.38	30.8	17.2	1.7
P (adjusted *v* ZB)		< .001			< .001		.025		NS		
ZBD	0.06	0.06	0.19	0.33	0.23	1.13	2.58	1.27	17.5	13.9	2.1
P (adjusted *v* ZB)		< .001			< .001		.002		.056		
ZBDK	0.03	0.02	0.11	0.24	0.13	0.82	1.61	1.04	19.0	15.1	1.4
P (adjusted *v* ZB)		< .001			.0017		.104		.091		

Abbreviations: AED, androstenedione; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; NS, not significant; RP, radical prostatectomy; SD, standard deviation; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.
*Androgen index is calculated as the sum of (5 × dihydrotestosterone concentration) + (1 × testosterone concentration).

dutasteride as compared with the percentage change from baseline in the control group (ZD, −88%; $P = .003$; ZBD, −86%; $P = .007$; ZBDK, −83%; $P = .049$; *v* ZB, −68%; Fig 3A, Appendix Table A1). Notably, the decreases in circulating testosterone, DHEA-S, and androsterone (a metabolite downstream of DHEA and AED) in the ZBDK cohort were greater than the declines observed in these androgens in the control arm, suggesting an effect of ketoconazole (−97% *v* −92%, $P = .055$ for testosterone; −67% *v* −12% for DHEA-S, $P = .007$; and −81% *v* −2%, $P < .001$ for androsterone; Figures 3B, 3C, and 3D).

Prostate Androgen Receptor Program Activity

To determine the effect of multitargeted AR pathway suppression on AR signaling, we evaluated prostate epithelial expression of AR and the androgen-regulated gene product, PSA, and TMPRSS2 by IHC (Fig 4). Consistent differences in expression among the three study arms were not observed (not shown); therefore, results from these cohorts were grouped and compared with tissues from untreated men and those receiving standard combined blockade (ZB).

Compared with untreated tissues, intense nuclear AR expression in benign prostate epithelium was markedly lower in the ZB

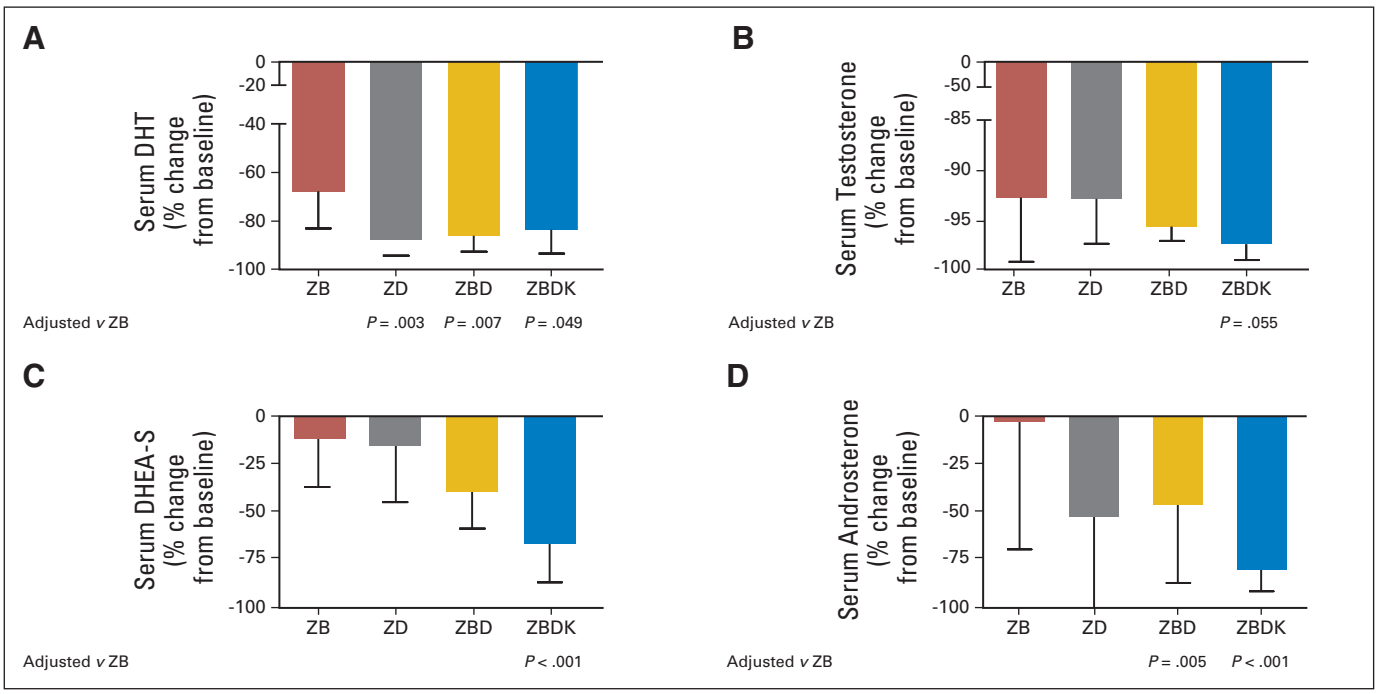

Fig 3. Change in serum androgen levels after 3 months of multitargeted neoadjuvant androgen suppression. The percentage change in androgen levels from baseline are depicted for each treatment group after 3 months of combined androgen blockade with goserelin and bicalutamide (ZB), goserelin and dutasteride (ZD), goserelin with bicalutamide and dutasteride (ZBD), and all three agents combined with ketoconazole (ZBDK). Levels of (A) dehydrotestosterone (DHT), (B) testosterone, (C) dehydroepiandrosterone sulfate (DHEA-S), and (D) androsterone were measured by mass spectroscopy in serum samples obtained before starting treatment and on the morning of prostatectomy. Differences between each treatment cohort and the control (ZB) group were evaluated by linear regression. Statistically significant *P* values ($P < .05$) or those trending toward significance ($P < .10$) are presented in italics below the relevant treatment group. No comparisons for DHEA or androstenedione were significant (data not shown).

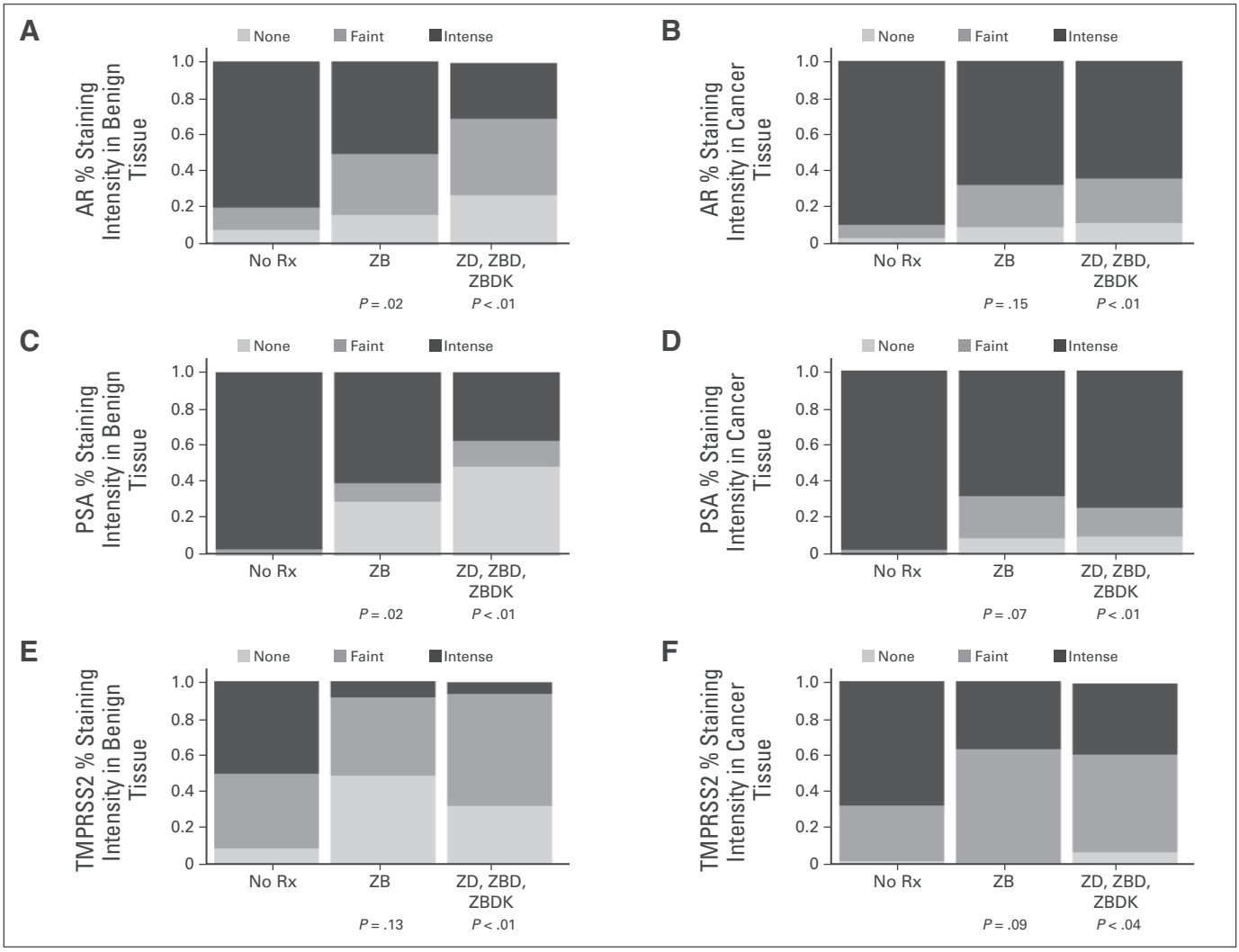

Fig 4. Immunohistochemical expression of androgen receptor (AR) and androgen-regulated genes after 3 months of multitargeted neoadjuvant androgen suppression. A tissue microarray comprising cores of benign and cancer tissue from each patient was analyzed for (A, B) nuclear expression of AR, and (C, D) cytoplasmic expression of prostate-specific antigen (PSA) and (E, F) TMPRSS2. Immunostaining was scored separately in benign (A, C, E) and cancer glands (B, D, F) using a compositional method based on the percentage of cells at each intensity level (0 for none, 1 for faint, and 2 for intense). The stacked bar graphs represent the proportion of cores in each treatment group that stain at the indicated intensity level (light gray, none; medium gray, faint; dark gray, intense). Differences between the indicated cohort and the untreated tissues were evaluated by unpaired *t* tests with Welch's correction. Statistically significant *P* values ($P < .05$) are presented in italics below the relevant treatment group. Rx, treatment; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.

and combined (ZD, ZBD, and ZBDK) study cohorts (Fig 4A; Appendix Table A2, online only). A trend for lower AR expression in the combined dutasteride cohorts compared with the ZB group was observed, but was not statistically significant. Although suppressed compared with untreated tissue, intense nuclear AR staining remained in the neoplastic epithelium of treated cohorts and was essentially identical in the ZB and the combined dutasteride arms (89% in untreated tissue compared with 62% [ZB, $P = .15$] and 64% [ZD/ZBD/ZBDK, $P = .01$]). The less significant *P* value for the ZB group likely reflects lower power as a result of fewer patients in the ZB subset compared with the grouped ZD/ZBD/ZBDK treatment arm.

Compared with untreated tissues, cytoplasmic expression of the AR-regulated gene products PSA and TMPRSS2 was substantially lower in benign prostate epithelium of both control ZB and combined dutasteride cohorts (Figs 4B and 4C; Appendix Table A2). Paralleling the changes in nuclear AR expression, PSA and TMPRSS2 staining in neoplastic glands was higher than in benign glands and expression was similar in both the ZB and ZD/ZBD/ZBDK groups.

PSA Nadir and Pathologic Outcomes

The median tumor volume at prostatectomy was lower in the ZBDK cohort, although the difference did not reach statistical significance (Fig 5A; Appendix Table A3, online only). The heterogeneity between cohorts and small numbers of patients compromise the ability to draw conclusions regarding clinical efficacy. There was one pathologic complete response in each of the ZBD and ZBDK cohorts, and nine (26%) of 34 patients in the combined study cohorts had small-volume residual disease, defined as tumor volume 0.2 cm^3 or less.[19] In prior studies, achieving a nadir PSA below 0.2 ng/mL in

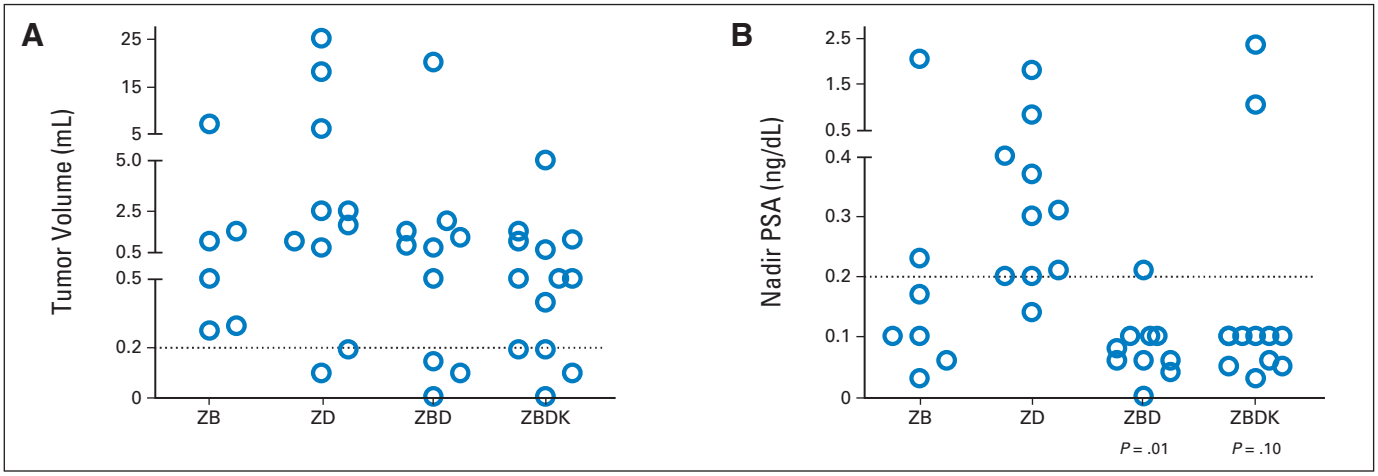

Fig 5. Distribution of tumor volume and prostate-specific antigen (PSA) nadir by treatment group. Tumor volume at (A) prostatectomy and (B) nadir PSA are depicted for each treatment group after 3 months of combined androgen blockade with goserelin and bicalutamide (ZB), goserelin and dutasteride (ZD), goserelin with bicalutamide and dutasteride (ZBD), and all three agents combined with ketoconazole (ZBDK). Dotted lines represent near complete response less than 0.2 cm^3 (A) and nadir PSA less than 0.2 ng/dL (B). *P* values for differences in treatment groups were assessed by two-sample tests of proportions.

response to androgen suppression was associated with improved clinical stage and tumor volume at prostatectomy and better survival in patients with relapsed disease[7,14,15] and is presumed to be related to nadir PSA reflecting greater tumor sensitivity to ADT. Fewer patients in the ZD group had nadir PSA less than 0.2 compared with the ZBD and ZBDK groups, (four of 10 *v* 10 of 10 and 10 of 12, respectively, $P = .01$ and $P = .10$; Fig 5B).

Notably, dichotomizing patients based on nadir PSA less than or ≥ 0.2 ng/mL demonstrated trends toward significantly lower serum testosterone, DHEA, and AED in patients with nadir PSA less than 0.2 ng/mL (Appendix Fig A1A, online only). The expression of AR, PSA, and TMPRSS2 in benign prostate epithelium was significantly lower in patients with PSA nadir less than 0.2 ng/mL; in contrast, expression of AR, PSA, and TMPRSS2 in cancer cells was identical regardless of serum PSA nadir (Appendix Figs A1B and A1C). These data demonstrate that although changes in serum PSA clearly associate with androgen-mediated effects in the prostate, these do not indicate cancer-associated suppression of androgen-mediated gene expression.

DISCUSSION

This study was designed to determine whether intraprostatic concentrations of testosterone and DHT could be suppressed below the levels achieved through standard ADT and whether improved suppression of androgens and AR signaling would enhance pathologic responses. We designed the study in 2006 to evaluate combinations of clinically available drugs that targeted distinct points of androgen metabolism or action. Compared with the group treated with goserelin and bicalutamide, the addition of dutasteride or dutasteride and ketoconazole resulted in substantially lower prostate DHT concentrations, more frequent PSA nadirs of less than 0.2, and more frequent pathologic complete (5.7%) and near-complete responses (20%). The heterogeneity of the treatment groups makes any conclusions regarding clinical response rate hypothesis generating, rather than definitive. The surrogacy of pathologic complete response or small-volume residual disease after neoadjuvant therapy has been clearly demonstrated in locally advanced breast cancer.[20-22] However, although the response rates observed in this study are promising and exceed rates reported in previous neoadjuvant trials of hormone suppression, longer follow-up in larger cohorts will be necessary before we can establish clinical relevance in prostate cancer.

Despite substantially reducing prostate DHT in the cohorts receiving dutasteride or ketoconazole, the combined activity of residual testosterone and DHT levels in the prostate remain at concentrations capable of activating the AR based on previous measurements studies using in vitro assays.[18] Although the absolute prostate or serum androgen levels were not lower in the ZBDK arm compared with the ZD or ZBD arms, the ZBDK group did show a statistically significant difference in the percentage decline of circulating testosterone, DHEA-S, and androsterone levels (particularly for DHEA-S) compared with the declines observed in the standard blockade arm (ZB). DHEA-S is the primary serum reservoir of DHEA, suggesting that in the absence of a CYP17A inhibitor, a significant depot of serum androgen precursors remain. Despite this evidence of activity with the addition of a CYP17A inhibitor, more substantial suppression of upstream precursors will be critical to minimizing tissue androgen levels and overall AR activation. In this context, the agents tested in this study should not be considered part of standard clinical practice.

Consistent with the mechanism of SRD5A inhibition, prostate tissue testosterone levels in all three dutasteride-containing cohorts were statistically higher than in men treated with combined blockade alone, even in the cohort receiving the CYP17A inhibitor ketoconazole. Similarly, AED levels were also significantly higher in tissues from dutasteride-treated patients. As AED is directly interconverted with testosterone, this may reflect metabolism of elevated testosterone to AED by HSD17B2. Alternatively, this could also be explained if AKR1C3 (mediating conversion of AED to testosterone) is rate-limiting, especially because AED is higher than testosterone. These data emphasize that androgen suppression strategies using SRD5A inhibition will require concomitant suppression of upstream androgen synthesis.

Notably, although the expression of AR, PSA, and TMPRSS2 was reduced in benign prostate epithelium and was significantly lower in

patients with PSA nadir less than 0.2 ng/m, the expression of these genes was readily detectable in cancer cells and showed no association with serum PSA nadir. These data suggest that changes in serum PSA clearly represent androgen-mediated effects. However, these most likely reflect the impact of suppressing androgen-mediated PSA secretion from benign prostate tissue and do not necessarily indicate cancer-associated suppression of androgen-mediated gene expression, PSA secretion, or tumor regression.

Important questions for future studies concern the variability in responses. Why a subset of patients had complete or near-complete tumor eradication, whereas substantial residual tumor volumes were found in others, remains to be determined. These tumors may survive androgen inhibition through mechanisms that include increased expression of AR or AR splice variants, the activity of androgen synthetic enzymes such as CYP17A or SRD5A, or through alternative signal transduction pathways.[23-30]

The AR fulfills an important criteria defining a lineage-survival oncogene—a master cell-type regulator on which neoplastic cells derived from prostate epithelium are dependent.[31] This study provides a template for evaluating the mechanisms underlying the efficacy of AR pathway-directed therapeutics. Through direct tissue assessments, our results indicate that treatment failure is likely still occurring via ineffective and incomplete AR pathway suppression. The neoadjuvant approach provides opportunities to rigorously test the hypothesis that extinguishing AR signaling—either through further ligand reduction and/or more effective direct AR targeting—will produce high rates of pathologic complete responses. Combining effective androgen synthesis inhibitors such as abiraterone with more potent AR antagonists such as enzalutamide is a reasonable strategy for assessing whether highly active antiandrogen therapy is an effective treatment strategy.[32]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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GLOSSARY TERMS

androgen receptor: A DNA-binding and hormone-activated transcription factor important to the development and progression of prostate cancer. Its primary ligand is dihydrotestosterone. In later-stage (castration-resistant) prostate cancer, oncogenic alterations such as androgen receptor overexpression allow the androgen receptor to continue signaling despite undetectable, or castrate, levels of serum testosterone.

PSA (prostate-specific antigen): A protein produced by cells of the prostate gland, the blood level of PSA is used as a tumor marker for men who may be suspected of having prostate cancer. Most physicians consider 0 to 4.0 ng/mL as the normal range. Levels of 4 to 10 and 10 to 20 ng/mL are considered slightly and moderately elevated, respectively. PSA levels have to be complemented with other tests to make a firm diagnosis of prostate cancer.

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Appendix

Table A1. Absolute Values and Mean Percentage Change in Post-Treatment Serum Androgen Levels

Treatment Category	DHT (ng/dL)		Testosterone (ng/dL)		AED (ng/dL)		DHEA (ng/dL)		DHEA-S (pg/dL)		Androsterone (ng/dL)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
No treatment	34.1	13.8	368	112	85.1	27.8	368	155	132.6	112.5	16.4	12.6
ZB, post-treatment	6.9	3.1	17.9	10.4	93.7	68.9	428	255	68.6	40.1	9.5	9.1
% change versus baseline	–68	12	–92	5	16	41	16	22	–12	3	–2	42
ZD, post-treatment	4.2	3.4	27.2	24.4	106	81.3	446	284	191.8	50.8	6.8	6.8
% change versus baseline	–88	8	–93	5	3	76	0	57	–16	19	–48	59
P (adjusted *v* ZB)	.0027		NS		NS		NS		NS		NS	
ZBD, post-treatment	3.6	2.0	13.4	4.3	66.8	33.7	272	205	53.3	55.7	7.6	7.1
% change versus baseline	–86	9	–95	2	–2	77	5	82	–40	23	–46	53
P (adjusted *v* ZB)	.0067		NS		NS		NS		NS		.005	
ZBDK, post-treatment	5.7	4.9	9.4	6.2	93.8	91.6	315	256	40.6	44.3	2.9	2.3
% change versus baseline	–83	15	–97	0.03	29	1.28	–7	77	–67	31	–81	15
P (adjusted *v* ZB)	.0492		.055		NS		NS		< .001		< .001	

Abbreviations: AED, androstenedione; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; NS, not significant; SD, standard deviation; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.

Table A2. Percentage of Tissue With High-Intensity IHC Expression of AR and Androgen-Regulated Genes in Benign and Malignant Prostate Epithelial Glands

Variable	Benign Prostate Epithelium			Malignant Prostate Epithelium		
	No Treatment (%)	ZB (%)	ZD, ZBD, ZBDK (%)	No Treatment (%)	ZB (%)	ZD, ZBD, ZBDK (%)
AR*	81	47	32	89	62	64
P v no Rx†		.02	< .01		.15	< .01
PSA*	99	49	42	97	65	74
P v no Rx†		.02	< .01		.14	< .01
TMPRSS2*	50	17	6	68	34	39
P v no Rx†		.13	< .01		.09	.04

Abbreviations: AR, androgen receptor; IHC, immunohistochemistry; PSA, prostate-specific antigen; Rx, treatment; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.

*AR, nuclear; PSA and TMPRSS2, cytoplasmic.

†*P* from Welch's two sample *t* tests.

Table A3. Post-Treatment PSA Nadir and Pathologic Stage at Prostatectomy in Control and Study Patients

Characteristic	Untreated Patients (n = 11)	Control Patients, ZB (n = 8)*	Study Patients		
			ZD (n = 12)	ZBD (n = 10)†	ZBDK (n = 13)
Nadir PSA ≤ 0.2	NA				
No.		5/7	4/10	10/10	10/12
%		85	40	100	83
Pathologic staging					
pT2	10	3	5	6	10
pT3 a/b	1	1/1	1/3	2/1	1/1
Tumor volume, n		6/6	10/11	10/12	13/13
Mean	1.9	1.8	5.8	2.7	0.9
Median	1.6	0.8	2.2	0.8	0.5
Pathologic response					
Complete (CR)	0	0	0	1	1
Near CR (≤ 0.2 cm^3)	0	0	2	2	3

Abbreviations: CR, complete response; PSA, prostate-specific antigen; ZB, goserelin and bicalutamide; ZBD, goserelin, bicalutamide, and dutasteride; ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole; ZD, goserelin and dutasteride.

*Two patients in this group underwent end-of-study biopsy instead of prostatectomy (one withdrew, one had radiation treatment).

†One patient in this group underwent end-of-study biopsy instead of prostatectomy (because of diagnosis of lung cancer).

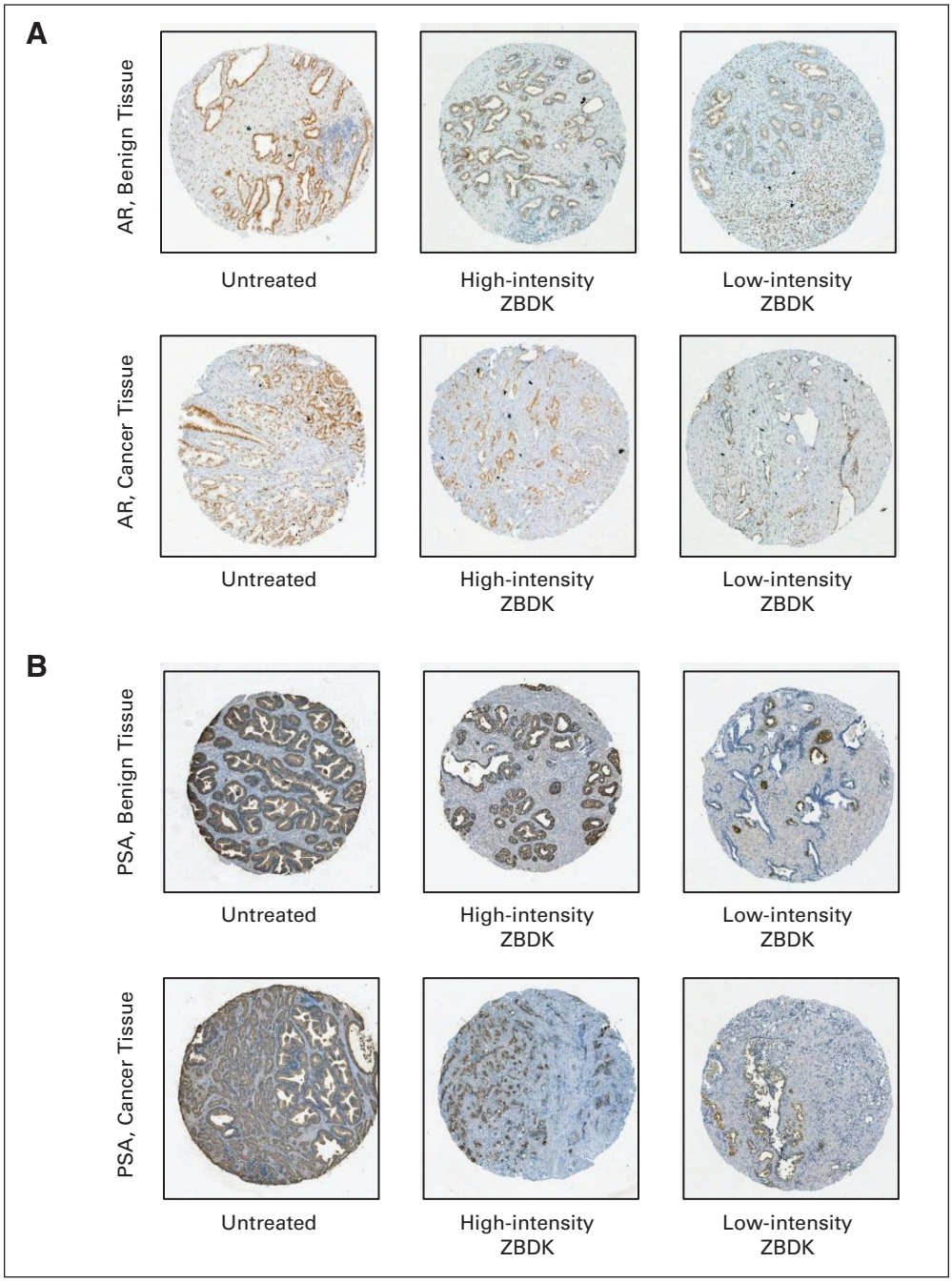

Fig A1. Prostatic expression of androgen receptor (AR) and prostate-specific antigen (PSA) after 3 months of multitargeted neoadjuvant androgen suppression. A tissue microarray comprising six cores of benign and cancer tissue from each patient was analyzed (A) for nuclear expression of AR and (B) for cytoplasmic expression of PSA in benign and malignant prostate epithelium. Representative examples of high- and low-intensity staining from different patients in the untreated and multitargeted treatment arms are shown for both benign and cancer cores. ZBDK, goserelin, bicalutamide, dutasteride, and ketoconazole .

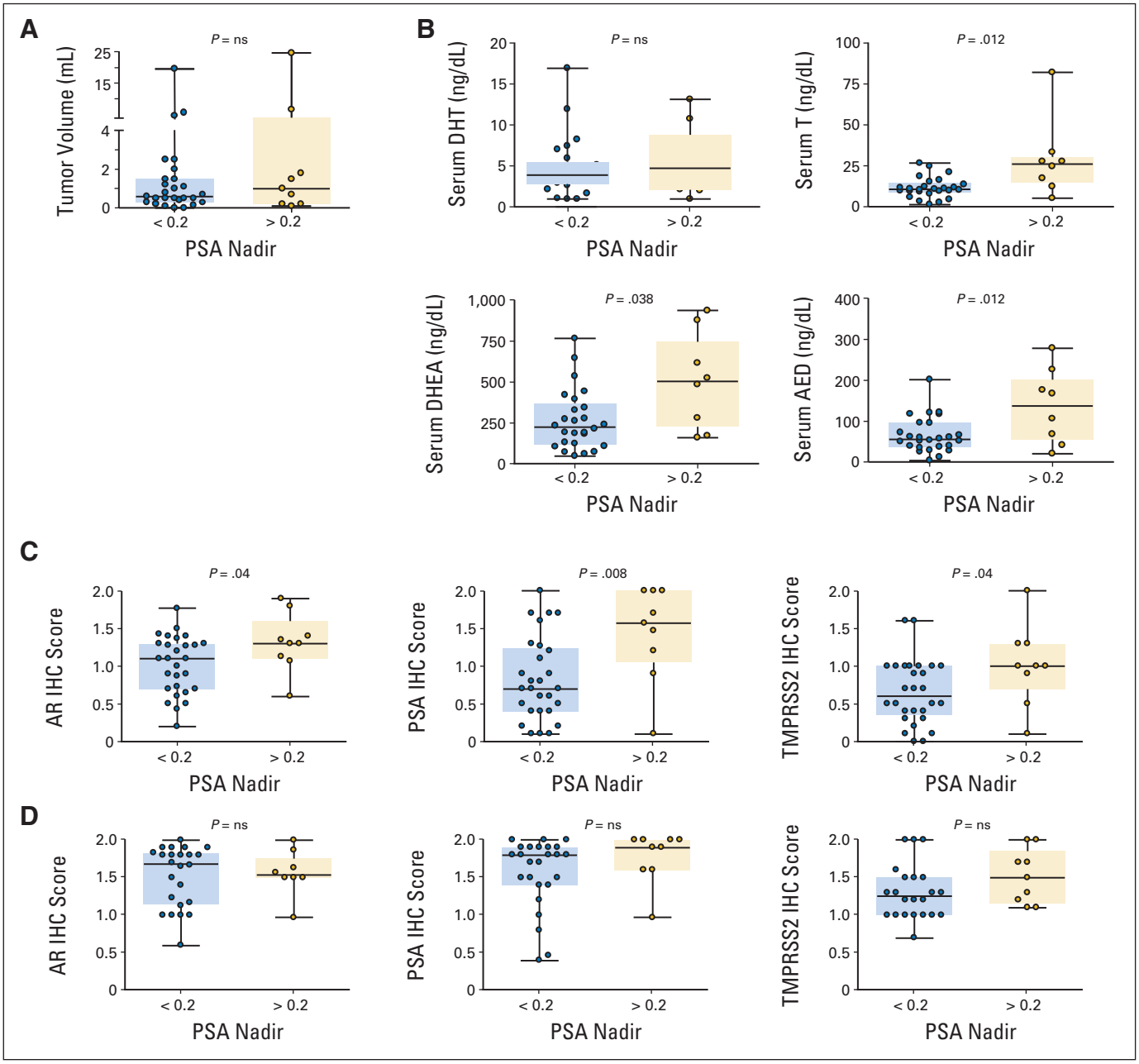

Fig A2. Association of prostate-specific antigen (PSA) nadir with tumor volume, serum androgens, and prostatic immunohistochemistry (IHC). Nadir PSA values were used to dichotomize all study patients into those with PSA nadir less than or more than 0.2 ng/dL to evaluate differences in (A) tumor volume, (B) serum androgen levels, and prostate IHC expression of androgen receptor (AR), PSA, and TMPRSS2 in (C) benign and (D) cancer tissue. Differences were evaluated by linear regression. Statistically significant *P* values ($P < .05$) or those trending toward significance ($P < .10$) are presented in italics below the relevant treatment group. AED, androstenedione; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.