Pharmacologic Basis for the Enhanced Efficacy of Dutasteride against Prostatic Cancers

Yi Xu, Susan L. Dalrymple, Robyn E. Becker, Samuel R. Denmeade, and John T. Isaacs

Abstract

Purpose: Prostatic dihydrotestosterone (DHT) concentration is regulated by precursors from systemic circulation and prostatic enzymes of androgen metabolism, particularly 5α-reductases (i.e., SRD5A1 and SRD5A2). Therefore, the levels of expression SRD5A1 and SRD5A2 and the antiprostatic cancer growth response to finasteride, a selective SRD5A2 inhibitor, versus the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, were compared.

Experimental Design: Real-time PCR and enzymatic assays were used to determine the levels of SRD5A1 and SRD5A2 in normal versus malignant rat and human prostatic tissues. Rats bearing the Dunning R-3327H rat prostate cancer and nude mice bearing LNCaP or PC-3 human prostate cancer xenografts were used as model systems. Tissue levels of testosterone and DHT were determined using liquid chromatography-mass spectrometry.

Results: Prostate cancer cells express undetectable to low levels of SRD5A2 but elevated levels of SRD5A1 activity compared with nonmalignant prostatic tissue. Daily oral treatment of rats with the SRD5A2 selective inhibitor, finasteride, reduces prostate weight and DHT content but did not inhibit R-3327H rat prostate cancer growth or DHT content in intact (i.e., noncastrated) male rats. In contrast, daily oral treatment with even a low 1 mg/kg/d dose of the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, reduces both normal prostate and H tumor DHT content and weight in intact rats while elevating tissue testosterone. Daily oral treatment with finasteride significantly ($P < 0.05$) inhibits growth of LNCaP human prostate cancer xenografts in intact male nude mice, but this inhibition is not as great as that by equimolar oral dosing with dutasteride. This anticancer efficacy is not equivalent, however, to that produced by castration. Only combination of dutasteride and castration produces a greater tumor inhibition ($P < 0.05$) than castration monotherapy against androgen-responsive LNCaP cancers. In contrast, no response was induced by dutasteride in nude mice bearing androgen-independent PC-3 human prostatic cancer xenografts.

Conclusions: These results document that testosterone is not as potent as DHT but does stimulate prostate cancer growth, thus combining castration with dutasteride enhances therapeutic efficacy.

Dihydrotestosterone (DHT) is the major intracellular growth factor for normal and neoplastic prostatic epithelial cells due to its high-affinity binding to androgen receptors (AR; ref. 1). The intracellular DHT concentration determines the prostatic cell content via its ability to regulate the proportion of ligand-occupied AR (2, 3). Once a critical threshold of AR is occupied by DHT, signal transduction pathways are activated which control the growth and functional activities of the prostatic epithelium (1, 3). The intracellular DHT concentration is thus paramount and regulated by both the supply of testosterone and other precursors from the systemic circulation and the complex interplay between intracellular prostatic enzymes of androgen metabolism, particularly the 5α-reductase (i.e., SRD5A) family of reductive enzymes that irreversibly convert testosterone into DHT (4). The SRD5A family includes two isoforms each encoded by a distinct gene (5). SRD5A1 (i.e., 5α-reductase type I) is expressed widely and is the major isoform expressed in tissues, such as liver and skin (5). Distinguishing characteristics of this type I isoform are its neutral (i.e., pH 7.0) optima and high (i.e., micromolar) K_m for testosterone (5). SRD5A2 (i.e., the type II isoform) is more restrictive in its expression, being the major isoform expressed by male sex accessory tissues, such as the prostate (5). Distinguishing characteristics of this type I isoform are its acidic (i.e., pH 5.0) optima and low (i.e., nmol/L) K_m for testosterone (5). Whereas the major isoform expressed in the prostate stroma is SRD5A2, normal and malignant prostate epithelial cells express SRD5A1 (5). Thus, DHT concentration in normal and neoplastic prostatic tissue is effected by varying inputs from both SRD5A1 and SRD5A2 isoforms.

A greater degree of suppression of the intracellular DHT concentration is required to inhibit the growth of malignant versus nonmalignant prostatic epithelial cells (2, 3, 6, 7). Thus,

Authors' Affiliation: Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Received 1/26/06; revised 4/25/06; accepted 4/28/06.

Grant support: NIH grant DK52645 and sponsored research agreement with GlaxoSmithKline.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: John T. Isaacs, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, Baltimore, MD 21231. Phone: 410-955-7777; Fax: 410-614-8397; E-mail: isaacjo@jhmi.edu.

doi:10.1158/1078-0432.CCR-06-0184

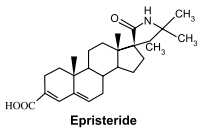
regimens that lower DHT concentrations sufficiently to decrease prostatic size and cellular content in nonmalignant prostatic tissue may not be adequate for therapeutic efficacy against prostate cancer. There are a variety of clinically approved methods for lowering tissue DHT, including luteinizing hormone–releasing hormone analogues, to suppress the systemic testosterone level as well as 5α-reductase inhibitors. Because 5α-reductase inhibitors do not inhibit the systemic testosterone level but instead target the production of DHT by SRD5A, their use as monotherapy was developed to allow selective tissue DHT suppression while maintaining or even enhancing tissue testosterone levels. In this way, the anabolic effects of testosterone on bone density, libido, and muscle mass are preserved while blocking pathologic growth-promoting effects of DHT.

Based on this rationale, orally active, steroidal 5α-reductase inhibitors, such as epristeride (8–11), finasteride (12–14), and dutasteride (refs. 14, 15; Table 1), were developed. At appropriate oral dose regimens, these agents lower DHT concentration sufficiently within prostates with benign prostatic hyperplasia to decrease gland size and relieve obstructive symptoms (16–20). Based on these results, finasteride and dutasteride are approved by the Food and Drug Administration for benign prostatic hyperplasia treatment. In addition, finasteride was the first 5α-reductase inhibitor tested as monotherapy for metastatic prostate cancer. The clinical efficacy of finasteride monotherapy in metastatic patients has been modest (21, 22). The reason for the limited effectiveness of finasteride may be due to the fact that although being a potent (i.e., IC_{50}, 69 nmol/L), time-dependent, irreversible inhibitor of the human SRD5A2 it is not as potent (i.e., IC_{50}, 360 nmol/L) or is not an irreversible inhibitor of the human SRD5A1 isoform (refs. 23, 24; Table 1). At clinical oral doses of 5 to 10 mg/d, maximum serum levels of finasteride are <200 nmol/L (13), which although above the IC_{50} for SRD5A2 is below its IC_{50} for the SRD5A1 isoform. Coupling this with its short serum half-life (Table 1) due to cytochrome *P*450–mediated oxidation of its *t*-butyl moiety (25) and its 82% binding to serum proteins (23) results in finasteride functionally being a SRD5A2-specific inhibitor (24). Human prostate cancers down-regulate their expression of SRD5A2 isoform while maintaining or even enhancing their expression of the SRD5A1 isoform (26–30). This raises the issue of whether a dual SRD5A1 and SRD5A2 inhibitor would be superior to inhibition of just the SRD5A2 isoform when treating such prostate cancer patients.

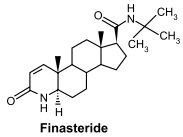
This possibility is supported by previous animal studies. Oral treatment of syngeneic Copenhagen male rats bearing androgen-responsive R-3327G rat prostatic cancers with the 5α-reductase inhibitor, epristeride, results in significant reduction of both G tumor growth and ventral prostatic (VP) cell content (i.e., 50% of the reduction in G tumor size and VP cell content produced by castration; ref. 7). This response correlated with suppression of DHT concentration in both G tumors and VP (7). Likewise, oral treatment with epristeride to nude mice bearing androgen-responsive PC-82 human prostate cancer xenografts results in tumor growth inhibition nearly identical to that produced by surgical castration (7). This growth inhibition in the PC-82 human cancers occurs, although such epristeride treatment elevated the PC-82 tumor testosterone concentration by 2-fold while lowering PC-82 tumor DHT concentration by 4-fold (7). These results documented that growth of prostate cancers are driven more by the concentration of tissue DHT than testosterone. This is consistent with DHT having a >10-fold higher potency for inducing AR signaling compared with testosterone (31). In contrast to this robust response of G and PC-82 prostate cancers, when rats bearing the androgen-responsive R-3327H rat prostate cancer are treated with a similar epristeride oral dose regimen, normal VP cell and DHT content is reduced but no inhibition of H tumor growth occurs and this inability is associated with a lack of suppression of H tumor 5α-reductase activity and DHT concentration (7). A similar inability to either inhibit H tumor growth or lower H tumor DHT concentration occurred when tumor-bearing rats were treated daily orally with

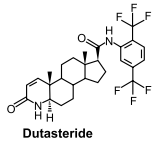
Table 1. Comparison of the serum half-life of various steroidal 5α-reductase inhibitors in rats versus humans and their species-specific inhibitory potency against SRD5A1 and SRD5A2 isoforms

Inhibitor (refs.)	Species	Serum half-life	Inhibitor potency (IC_{50}), nmol/L	
			SRD5A1 isoform	SRD5A2 isoform
Epristeride (8–11)	Rat	3 h	20 ± 7	11 ± 1
	Human	27 h	350 ± 50	14.6 ± 2.4
Finasteride (12–14)	Rat	2 h	5.4 ± 0.2	0.5 ± 0.1
	Human	6 h	360 ± 40	69 ± 1
Dutasteride (14, 15)	Rat	31 h	0.3 ± 0.02	0.2 ± 0.04
	Human	>3 d	6 ± 1	7 ± 3



Epristeride


Finasteride


Dutasteride

finasteride at a dose that reduced normal VP cell and DHT content (7, 32). These results document that H tumors express SRD5A activity not inhibited by type 2 specific inhibitors.

The 5α-reductase inhibitor, dutasteride, is identical to finasteride, except in position 17 (Table 1). In dutasteride, the carbamoyl group at position 17 is coupled to a bis(trifluoromethyl)-phenyl moiety instead of a *t*-butyl moiety. This modification shifts the serum half-life and increases the inhibition potency of dutasteride as a reversible SRD5A1 inhibitor and a time-dependent, irreversible SRD5A2 inhibitor (ref. 23; Table 1). Due to its longer serum half-life and lower IC_{50} for both SRD5A1 and SRD5A2 (Table 1), dutasteride at clinical oral doses of 5 mg/d, which produces steady-state serum drug levels of $\geq$1 μmol/L (20), is a dual 5α-reductase inhibitor. Therefore, a series of models were used to determine whether there is an enhanced efficacy when dutasteride is used to inhibit both SRD5A1 and SRD5A2 for the treatment of prostate cancer.

Materials and Methods

Reagents. The 5α-reductase inhibitor dutasteride [17β-*N*-(2,5-bis (trifluoromethyl)phenyl-carbamoyl)-4-aza-5α-androst-1-en-3-one] was provided by GlaxoSmithKline (Research Triangle Park, NC), and finasteride [17β-(*N*-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one] was obtained from Kemprotec Ltd. (Middlesbrough, United Kingdom). For oral dosing, animals received daily gavage with 200 μL polyethylene glycol 400/1% Tween 80 (Sigma; St. Louis, MO) as vehicle containing indicated dose of drug.

Tissues, tumors, and cell lines. Nonmalignant human prostate tissue was obtained from radical prostatectomy specimens from seven patients undergoing surgery for localized prostate cancer under an institutional review board–approved protocol. The rat tumors used (i.e., H and G sublines) are members of the Dunning R-3327 system of serially transplantable rat prostatic cancers whose developmental history and characteristics have been described (33). The source and characteristics for all of the normal and malignant human prostate cell lines used have been described (34, 35). *In vitro* growth responses to various agents was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described (35).

Real-time Taqman reverse transcription-PCR quantitation of SRD5A1 and SRD5A2 expression. The Taqman primers and probes for human and rat SRD5A1 and SRD5A2 genes were purchased from Applied Biosystems (Foster City, CA). Total RNA was extracted with Qiagen RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The reverse transcription and subsequent polymerase reactions were done using appropriate Taqman reagents and protocol using a Bio-Rad ICycler (Bio-Rad, Hercules, CA). Each reverse transcription-PCR experiment included a standard curve of known copy number using full-length wild-type cDNA of SRD5A1 and SRD5A2 for either rat or human subcloned into the TA-cloning vector (Invitrogen, Carlsbad, CA). Using the appropriate species-specific primers and copy number standards, SRD5A1 and SRD5A2 mRNA levels in the rat or human tissues were determined and expressed in copy number per microgram of total RNA.

5α-Reductase enzymatic assays. The determination of SRD5A1 and SRD5A2 isoform-specific enzymatic activities was achieved by subtracting the SRD5A1 isoform-specific activity from the total 5α-reductase activity for each tissue. The SRD5A1 isoform-specific activity was determined by irreversibly inhibiting the SRD5A2 activity selectively within tissue homogenates using a preincubation step with NADPH in the presence of finasteride at a concentration of 5 nmol/L for rodent tissues or 100 nmol/L for human tissues. This assay is based on the studies of Azzolina et al., which documented that within 1 hour of incubation with 0.5 mmol/L NADPH and these concentrations of finasteride, SRD5A2 isoform is completely and irreversibly inhibited (36). In addition, this group documented that the subsequent dilution of finasteride to 0.5 nmol/L for rat tissues and 10 nmol/L for human tissue does not allow reactivation of the inhibited SRD5A2 isoform but is too low a concentration to inhibit the SRD5A1 isoform (36). Based on these results, 1:1 wet weight/volume whole (unfractionated) cell homogenates were produced with an all-glass tissue homogenizer (Kontes, Inc., Vineland, NJ) using 0.1 mol/L sodium phosphate (pH 6.6) containing 0.5 mmol/L NADPH (i.e., H buffer). Two separate 100 μL aliquots were processed: (*a*) with no finasteride pretreatment for the determination of the total 5α-reductase (i.e., SRD5A1 plus SRD5A2) activity and (*b*) with a 1-hour finasteride (i.e., 5 nmol/L for rat tissues or 100 nmol/L for human tissues) and 0.5 mmol/L NADPH preincubation at 37°C for the selective determination of SRD5A1 only. Both of the aliquots were then diluted with the addition of 400 μL H buffer. A 100 μL aliquot for each of these diluted mixtures was then incubated at 37°C with 100 μL H buffer containing 0.5 mmol/L NADPH and 0.1 μmol/L [^{3}H]testosterone (Amersham; Piscataway, NJ; specific activity, 50 Ci/mmol) to give a final concentration of 0.5 mmol/L NADPH and 50 nmol/L testosterone. At 30 and 60 minutes, 30 μL of the mixtures were processed by TLC to determine the picomoles of 5α-reduced products found per hour per 10^8 cells as described previously (7).

Animal studies. All animals used in these studies were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the specific protocols used were approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Adult male inbred Copenhagen rats (175-200 g body weight) were used for serial passage of the R-3327H and R-3327G rat prostatic cancer lines and 4- to 6-week-old male athymic nude BALB/c *nu/nu* mice were used for passage of the PC-82, LNCaP, and PC-3 human prostate cancer variants as described previously (7). All animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN).

For the testing of the *in vivo* antitumor growth response of Dunning R-3327H rat prostatic cancers to 5α-reductase inhibitors, Copenhagen male rats were inoculated s.c. in the flank with 20 mg minced H tumor in 0.5 mL Matrigel (Collaborative Biomedical, Bedford, MA). When the H tumors reached 1 to 2 cm^3 in size, randomization was done into groups of 10 tumor-bearing animals each and the groups were given various treatments. Tumor volumes were recorded each 5 to 7 days using microcalipers to determine the volume of the tumors as described previously (7). After 55 days of treatment, blood was drawn and VP and H tumors were removed and weighed.

For testing against the LNCaP and PC-3 human prostate cancer, male nude mice were inoculated in the flank with 200 μL Matrigel containing 2×10^6 viable LNCaP or PC-3 cells harvested from exponentially growing *in vitro* cultures. When the tumors reached a starting size of 100 mm^3, randomization was done into groups of 10 tumor-bearing animals each and the groups were given various treatments. After the indicated time of treatment, the tumor weights were determined for each animal.

Serum and tissue DHT and testosterone determination. The blood and tissues were extracted and extracts were assayed for determination of their testosterone and DHT levels using a validated tandem mass spectrometry method by PPD-Pharmaco (Richmond, VA).

Statistical analysis. Data (mean ± SE) were analyzed by one-way ANOVA. $P < 0.05$ is considered significant.

Results

Expression of SRD5A1 and SRD5A2 in normal and malignant prostate tissues. SRD5A2 mRNA expression is 2-fold higher than SRD5A1 in the normal rat and 4-fold higher in human prostatic tissue (Fig. 1A). SRD5A1 mRNA is detectable in early-passage cultures of nonimmortalized normal human prostatic epithelial (i.e., PREC) cells and in three different immortalized

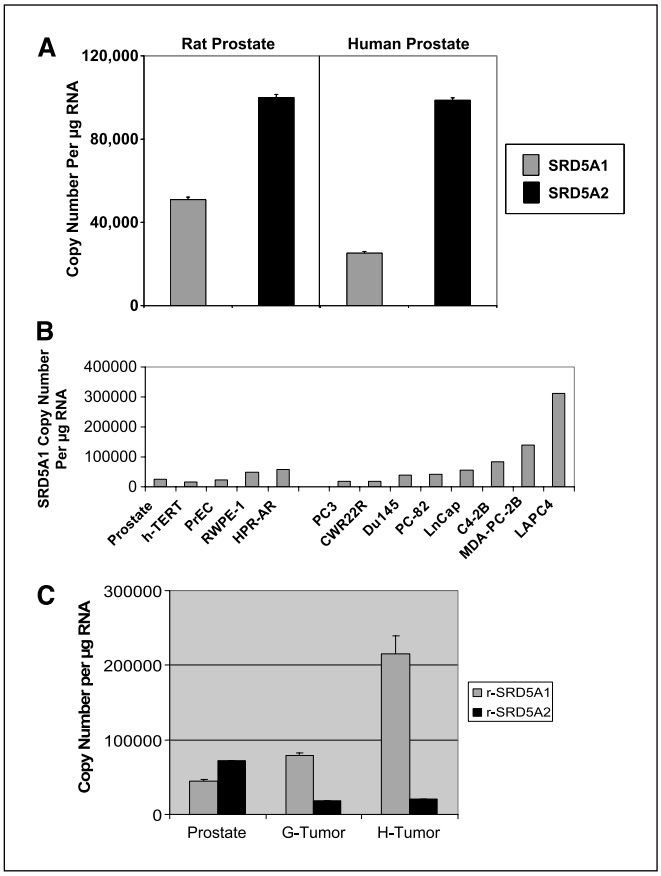


Fig. 1. Expression for SDR5A1 versus SDR5A2 mRNA in (*A*) normal rat VP and human prostatic tissue, (*B*) a series of nonmalignant human prostatic cell lines (i.e., 957e/hTERT, PREC, RWPE-1, and HPR-AR) versus malignant human prostatic cancer cell lines (i.e., PC-3, CWR22Rv1, DU145, PC-82, LNCaP, MDA-PC-2B, and LAPC-4), and (*C*) normal rat VP tissue versus Dunning R-3327G and R-3327H variants rat prostate cancer tissue. *Columns,* mean copy number/μg RNA; *bars,* SE.

normal human prostatic cell lines (i.e., hTERT, RWPE-1, and HPR-AR) at levels comparable with that expressed by normal human prostatic tissue (Fig. 1B). Although SRD5A1 mRNA is expressed in all malignant human (i.e., PC-3, CWR22Rv1, MDA-PC-2B, C4-2B, DU145, LAPC-4, PC-82, or LNCaP; Fig. 1B) and rat (i.e., H and G; Fig. 1C) prostate cancer variants, expression is significantly elevated in several of these (i.e., MDA-PC-2B and LAPC-4 human and H rat prostatic cancer). In contrast, normal rat and human prostatic tissues and H and G rat prostate cancer tissues contain detectable levels of SRD5A2, whereas none of the human normal or malignant cell lines do. This is because these tissue samples, unlike the cell lines, contain supporting host stromal cells and such stromal cells express SRD5A2 (5). As further documentation that the SRD5A2 mRNA level detectable from tissue samples is derived from contaminating stromal not epithelial cells, reverse transcription-PCR analysis was done on a pure population of G rat prostate cancer cell line exponentially growing in cell culture. When these *in vitro* cultured G cells were analyzed for SRD5A1 and SRD5A2 expression, the level of SRD5A1 mRNA remains high, whereas SRD5A2 is undetectable.

SRD5A1 versus SRD5A2 isoform-specific enzymatic activity. These mRNA results document that the SRD5A1 is the major, if not exclusive, isoform expressed by malignant rat and human prostate cancer cells. Previous studies showed that SRD5A mRNA levels are correlated with 5α-reductase activity (37, 38). In these previous studies, the 5α-reductase enzymatic assays were done at both acidic and neutral pH in an attempt to evaluate the activity associated with SRD5A1 versus SRD5A2 isoforms. Unfortunately, Smith et al. (39) documented that the pH profile of the SRD5A1 versus SRD5A2 is both too broad and varies depending on the concentration of testosterone used in the assay [i.e., at 20 nmol/L testosterone concentration, SRD5A2 has a pH 5.0 (optima) that is shifted to an optima at pH 6.5 at 1 μmol/L testosterone concentration] to allow accurate determination based on pH alone. An accurate determination of the isoform-specific activities is possible if a preincubation treatment with the appropriate concentration of finasteride is used to selectively, and irreversibly, inhibit SRD5A2 activity so that the SRD5A1 isoform-specific activity can be accurately determined. By subtracting this SRD5A1 isoform-specific activity from the total (i.e., SRD5A1 + SRD5A2) activity assayed without finasteride treatment, the SRD5A2 isoform-specific activity can be determined.

Using this method, the SRD5A1 or SRD5A2 activity was determined from a series of normal or malignant rat and human prostatic tissues harvested directly from their host without cell culturing. These results documented that the level of SRD5A2 enzymatic activity is >10-fold higher than SRD5A1 enzymatic activity in the normal rat VP (Table 2). These enzymatic activity differences are thus higher than predicted by the 2-fold difference in mRNA expression (Fig. 1A). In contrast, the SRD5A2 activity in the H and G rat prostate cancer tissues derived from contaminating tumor stromal cells is >90% reduced compared with normal rat prostate (Table 2). Again, this is a greater reduction in activity than predicted by mRNA levels (Fig. 1C). For the H rat prostate cancer, this reduction in SRD5A2 activity is coupled to a >5-fold increase in SRD5A1 activity (Table 2), which is consistent with the comparative mRNA levels (Fig. 1C). Such an enhancement in SRD5A1 activity was not observed for the G rat prostate cancer (Table 2).

With regard to human tissues, the SRD5A2 activity is >10-fold higher than SRD5A1 activity in nonmalignant human prostate tissue (Table 2), which is higher than predicted based on mRNA expression data (Fig. 1A). Both xenografted PC-82 and LNCaP tumor tissue express a low level of SRD5A2 activity

Table 2. Level of enzymatic activity of SRD5A1 and SRD5A2 isoforms in various tissues harvested directly from hosts with no cell culturing

	5α-Reductase activity (pmol/h/10^8 cells)	
Tissue (n = 3-5)	**SRD5A1**	**SRD5A2**
Rat VP	30 ± 5	391 ± 21
R-3327H rat prostatic cancer	164 ± 21*	31 ± 9*
R-3327G rat prostate cancer	35 ± 3*	15 ± 4*
Human prostate tissue	48 ± 7	545 ± 75
PC-82 human prostate cancer	20 ± 6†	10 ± 5†
LNCaP human prostate cancer	104 ± 34†	18 ± 2†

*$P < 0.05$ versus rat prostate.
†$P < 0.05$ versus human prostate.

(Table 2), derived from contaminating tumor stromal cells. SRD5A1 activity was low in the PC-82 but elevated 2-fold in the LNCaP prostate cancer tissue compared with nonmalignant prostatic tissue (Table 2).

Pharmacologic basis for selective efficacy of finasteride and dutasteride for normal rat prostate versus H rat prostate cancer. Because SRD5A1 enzymatic activity is high in the R-3327H rat prostate cancer tissue and not in the normal rat prostate, R-3322G rat prostate, or PC-82 human prostate cancer (Table 2), this could explain why finasteride suppress the tissue DHT concentration in these latter tissues but not in the R-3327H rat prostate cancers. This suggests that a dual 5α-reductase inhibitor with a longer serum half-life and/or higher potency is needed to suppress the DHT concentration in R-3327H rat prostatic cancers. Therefore, rats s.c. bearing 1 to 2 cm^3 R-3327H rat prostatic cancers were randomized into receiving daily oral b.i.d. treatment with varying doses of either finasteride or dutasteride and the effect on tumor volume was monitored for 55 days of treatment. The choice of dose range used is based on pharmacokinetic modeling that the nadir level of serum finasteride produced by multiday oral dosing is 0.5 nmol/L at 0.7 mg/kg/d (i.e., 1.89 nmol/kg/d), 5.4 nmol/L at 7 mg/kg/d (i.e., 18.9 nmol/kg/d), and 54 nmol/L at 70 mg/kg/d (i.e., 189 nmol/kg/d; ref. 22). Due to its >10-fold longer half-life, similar pharmacokinetic modeling predicts the equimolar dosing with dutasteride should produce nadir serum values of 125 nmol/L at 1 mg/kg/d (i.e., 1.89 nmol/kg/d), 1.25 μmol/L at 10 mg/kg/d (i.e., 18.9 nmol/kg/d), and 12.5 μmol/L at 100 mg/kg/d (i.e., 189 nmol/kg/d; ref. 22). Experimental confirmation of the accuracy of these modeling data is provided by the observation that oral dosing of rats with 1 mg/kg/d produced peak serum dutasteride levels of 263 nmol/L (40).

As controls, groups of H rat prostate cancer–bearing animals were dosed b.i.d. orally with vehicle or surgically castrated and then given oral dosing b.i.d. of vehicle. These studies document that the R-3327H rat prostate cancer essentially stops growth following surgical castration (Fig. 2). This response to castration is correlated with a >90% decrease in serum testosterone and H tumor tissue concentration of both testosterone and DHT (Table 3). Likewise, the concentration of testosterone and DHT decreases by >90% within the VP of such castrated animals. This is correlated with a 70% reduction in the weight of the VP of the castrated animals. Treatment with finasteride, even at doses of 70 mg/kg/d (189 nmol/kg/d), is unable to decrease the growth of the H rat prostate cancers (Fig. 2) due to its inability to lower H tumor DHT concentration (Table 3). In contrast to the situation for the H tumor, the weight and DHT concentration of VP, which expresses only a low level of SRD5A1 (Table 2), is significantly decreased in these tumor-bearing rats treated with finasteride (Table 3). In fact, there is a dose-response relationship between amount of finasteride and decreases in both prostatic weight and prostatic DHT concentration (Table 3). When tumor-bearing animals are treated with dutasteride, not only did the VP weight decrease, but also there is also a significant decrease in H tumor growth (Fig. 2) and the tumor weight at the end of 55 days of treatment (Table 3). This antitumor effect is associated with a dose-response reduction in DHT content coupled with a >2-fold increase in serum testosterone and tissue testosterone levels in both H tumor and VP (Table 3). Once the DHT content in the H tumor is reduced by 75% to ≈3 nmol/L by the lowest dosing of dutasteride, however, there is no further anticancer growth inhibition produced by the higher doses, although the highest dose reduces DHT content by an additional 90% (Table 3). These results document that although the highest dutasteride dose reduces H tumor DHT to the essential same nadir produced by castration it does not produce the same magnitude of prostate cancer growth inhibition. This is consistent with an elevated tumor testosterone level stimulating prostate cancer growth.

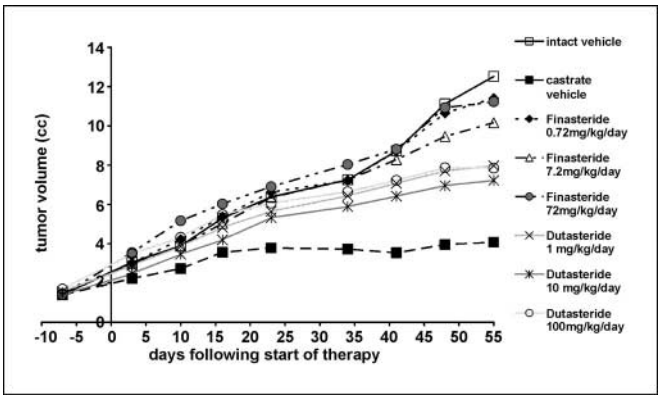


Fig. 2. Growth response of R-3327H rat prostate cancers in intact male rats treated orally b.i.d. with indicated dose of finasteride versus dutasteride for 55 days. Tumor-bearing rats were allowed to go untreated until the cancers were 1 to 2 cm^3 and were randomized into groups of 10 and begun on indicated treatments. For comparison, the tumor growth response in intact rats treated orally b.i.d. with vehicle or castrated and treated orally b.i.d. with vehicle was also determined. *Points,* mean; SE was omitted for clarity because it was ≤10% for each time point.

In vivo *response of LNCaP human prostate cancer cells to 5α-reductase inhibition alone and in combination with androgen ablation.* Based on the ability of testosterone to stimulate prostate cancer growth, the efficacy of dual SRD5A inhibition, theoretically, should be enhanced by simultaneously lowering the tumor testosterone levels by androgen ablation. As a model system to evaluate this possibility, the LNCaP cell line was used because it expresses 2-fold higher levels of both SRD5A1 mRNA (Fig. 1B) and enzymatic activity compared with nonmalignant human prostate tissue (Table 2). In addition, growth of established (i.e., 100 mm^3) LNCaP prostate cancers in male nude mice is profoundly inhibited by androgen ablation (i.e., castration) to lower serum and tumor testosterone levels (Fig. 3). Therefore, intact male nude mice bearing (100 mm^3) LNCaP prostate cancers were left either intact or castrated and treated for 28 days with equimolar daily oral dosing of finasteride (i.e., 70 mg/kg/d b.i.d.) or dutasteride (i.e., 100 mg/kg/d b.i.d.; Fig. 3). Whereas finasteride significantly decreases LNCaP prostate cancer growth, dutasteride is superior in its anticancer efficacy at an equimolar dose. Castration, however, produces a greater reduction in subsequent LNCaP cancer growth compared with either finasteride or dutasteride monotherapy. As predicted, however, only the combination of dutasteride treatment with castration produces a greater ($P < 0.05$) tumor growth inhibition than castration monotherapy alone (Fig. 3).

Specificity of antiprostatic cancer responsiveness to dutasteride. Lazier et al. reported that dutasteride at concentrations of ≥50 μmol/L kills both AR-positive androgen-sensitive LNCaP and AR-negative androgen-independent PC-3 cells in culture in a manner not reversed by DHT supplementation (41). Because

Table 3. Effect of castration versus 5α-reductase inhibitors on serum testosterone in VP and R-3327H prostate cancer tissue testosterone, DHT, and wet weight

Treatment (n = 10 rats per group)	Oral dose (mg/kg/d)*	Serum testosterone (nmol/L)	VP tissue			H tumor tissue		
			Testosterone (nmol/L)	DHT (nmol/L)	Weight (mg)	Testosterone (nmol/L)	DHT (nmol/L)	Weight (g)
Intact	—	12.8 ± 3.8	8.1	21.6 ± 2.9	143 ± 11	9.3 ± 0.9	12.3 ± 0.8	7.9 ± 1.1
Castrate	—	≤0.1	≤0.2†	≤0.2†	45 ± 7†	0.1 ± 0.1†	0.1 ± 0.1†	1.9 ± 0.2†
Finasteride	0.7 (1.89)	13.1 ± 3.1	20.9†	6.3†	88 ± 7†	11.9 ± 1.5	8.8 ± 1.0	7.5 ± 0.8
	7 (18.9)	27.3 ± 5.8†	29.0†	3.7†	71 ± 6†	18.8 ± 1.0†	6.9 ± 0.7	6.2 ± 1.0
	72 (189)	9.7 ± 2.1	19.9†	2.7†	67 ± 5†	13.8 ± 2.0	6.4 ± 0.9	7.0 ± 1.0
	1 (1.89)	24.6 ± 6.6†	27.0†	0.8†	74 ± 7†	19.9 ± 2.2†	3.2 ± 0.5†	5.2 ± 0.7†
Dutasteride	10 (18.9)	24.6 ± 5.5†	25.9†	0.2†	74 ± 4†	20.4 ± 1.7†	0.9 ± 0.1†	4.9 ± 0.5†
	100 (189)	21.7 ± 3.8†	22.5†	<0.1†	69 ± 5†	16.7 ± 1.9†	0.3 ± 0.1†	4.8 ± 0.8†

*Given as a divided dose, 12 hours apart for 55 days. Number in parentheses is the dose expressed in μmol/kg/d.
†$P < 0.05$, compared with intact control value.

the 100 mg/kg/d oral dose of dutasteride used for the LNCaP model produces serum dutasteride levels of ≈12.5 μmol/L (22), this raises the issue of whether at this dose the efficacy of dutasteride alone or in combination with androgen ablation involves its specific ability to inhibit SRD5A and/or a general toxic effect on cell survival. To resolve this, intact male nude mice were inoculated with PC-3 cells and allowed to go untreated until the cancers reached 100 mm^3 and then randomized into four groups of 10 tumor-bearing mice each. One group each was (*a*) given b.i.d. daily dosing with vehicle only (i.e., intact controls), (*b*) given b.i.d. daily dosing with 100 mg/kg/d dutasteride, (*c*) castrated and given b.i.d. dosing with vehicle (i.e., castrate controls), or (*d*) castrated and given b.i.d. dosing with dutasteride. After 14 days of treatment, there was no difference in the size of the PC-3 cancers in any of the four groups (i.e., cancers were 395 ± 20 mm^3 in intact controls, 375 ± 45 mm^3 in castrate controls, 410 ± 39 mm^3 in intact mice given dutasteride, and 400 ± 31 mm^3 in castrated mice given dutasteride). These results are consistent with a requirement for the prostate cancer being AR positive and sensitive to its signaling for it to be responsive to 100 ng/kg/d dutasteride.

Discussion

The present studies showed that prostate cancer cells express only a low level of SRD5A2 mRNA and enzymatic activity but often express enhanced levels of SRD5A1 activity compared with nonmalignant prostate tissue. These observations have significant implications for the use of 5α-reductase inhibitors for prostate cancer. 5α-Reductase inhibitors do not suppress the systemic levels of testosterone and their long-term use does not produce muscle, bone, or libido loss, although such treatments lower prostatic DHT inducing regression of nonmalignant prostatic tissue (17, 19). These clinical observations document that regulation of nonmalignant prostatic epithelial survival and proliferation is primarily driven by prostatic DHT and by not testosterone levels. This is because SRD5A2 enzymes in the prostate stroma produce an adequate level of DHT to occupy a sufficient number of AR within the nuclei of prostate stromal cells to induce their production of a critical level of specific paracrine peptide growth factors (termed andromedins; refs. 1, 4). Once formed in the stromal compartment, these paracrine andromedins diffuse into the epithelial compartment where they bind to their cognate receptors in the basal and luminal cells regulating their proliferation and survival, respectively (1, 4). If sufficient inhibition of stromal SRD5A2 is produced to prevent adequate production of DHT and thus stromal andromedins,

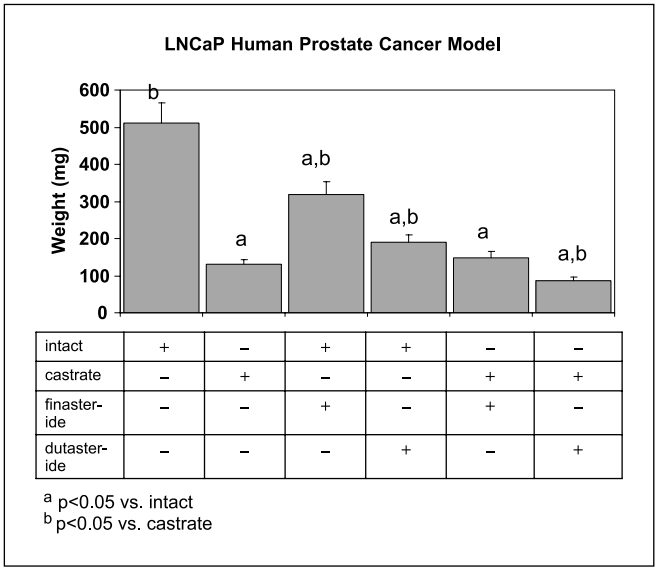


Fig. 3. Growth response of LNCaP human prostate cancers xenografts in mice given equimolar b.i.d. oral dosing with finasteride (i.e., 70 mg/kg/d or 189 nmol/kg/d) versus dutasteride (i.e., 100 mg/kg/d or 189 nmol/kg/d) alone or in combination with castration. Tumor-bearing mice were allowed to go untreated until the cancers were 100 mm^3 and then randomized into groups of 10 tumor-bearing mice each and begun on indicated treatment. For comparison, the tumor growth response in a group of intact or castrated mice receiving vehicle only was also determined.

then regression of the prostatic epithelium is induced (7). This stromal-based paracrine AR axis in nonmalignant prostate tissue explains why a SRD5A2 selective inhibitor, such as finasteride, can produce nonmalignant prostatic regression and can be used for treatment of benign prostatic hyperplasia, although it does not inhibit SRD5A1 expressed within prostatic epithelial cells.

During prostatic carcinogenesis, molecular changes occur producing a gain of function in the AR axis from a paracrine to an autocrine pathway in which occupancy of the AR by DHT within the nuclei of malignant cells directly controls the autocrine production of growth factors for survival and proliferation of these malignant cells (1, 42). Coupled with these changes, there is enhanced expression of the SRD5A1 isoform by prostate cancer cells, enhanced SRD5A1 expression is consistent with why previous clinical trials with the SRD5A2-specific inhibitor, finasteride, for the treatment of metastatic prostate cancer have had limited success either when used as monotherapy (21, 22) or when combined with antiandrogens (43). In the present studies, the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, has been documented to have enhanced *in vivo* efficacy compared with a SRD5A2-specific inhibitor against both rat and human prostate cancers. This efficacy is not equivalent as monotherapy, however, to that produced by standard androgen ablation alone. These results document that although testosterone is not as potent as DHT in driving malignant growth it still does stimulate such growth. The present experimental studies document that a combination of dual SRD5A1 and SRD5A2 inhibition with standard androgen ablation produces at least an additive antitumor growth-inhibitory effect compared with either monotherapy alone. The rationale for such an enhancement is that standard androgen ablation decreases testicular testosterone production but does not completely eliminate prostate cancer tissue testosterone content (44). Thus, in the androgen-depressed environment induced by standard androgen ablation, local 5α-reduction within metastatic prostate cancer cells amplifies the remaining androgen signal. Such androgenic amplification can be inhibited by simultaneous treatment with a dual 5α-reductase inhibitor, such as dutasteride. This dutasteride-androgen ablation combinational approach while limiting the amplification of androgen does not eliminate all AR-dependent signaling. This suggests that to block remaining AR growth signaling a novel (i.e., "bulky") antiandrogen may need to be added to the dutasteride-androgen ablation combination (45). Presently, we are developing such novel monofunctional and bifunctional "bulky" antiandrogens for such combinatorial AR-targeted therapy.

References

1. Litvinov IV, De Marzo AM, Isaacs JT. Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 2003;88:2972–82.
2. Ellis WJ, Isaacs JT. Effectiveness of complete versus partial androgen withdrawal therapy for the treatment of prostatic cancer as studied in the Dunning R-3327 system of rat prostatic adenocarcinomas. Cancer Res 1985;45:6041–50.
3. Kyprianou N, Isaacs JT. Quantal relationship between prostatic dihydrotestosterone and prostatic cell content: critical threshold concept. Prostate 1987;11:41–50.
4. Isaacs JT. Role of androgens in prostatic cancer. Vitam Horm 1994;49:433–502.
5. Russell DW, Wilson JD. Steroid 5α-reductase: two genes/two enzymes. Annu Rev Biochem 1994;63:25–61.
6. van Weerden WM, van Steenbrugge GJ, van Kreuningen A, Moerings EP, de Jong FH, Schroder FH. Assessment of the critical level of androgen for growth response of transplantable human prostatic carcinoma (PC-82) in nude mice. J Urol 1991;145:631–4.
7. Lamb JC, Levy MA, Johnson RK, Isaacs JT. Response of rat and human prostatic cancers to the novel 5α-reductase inhibitor, SK&F 105657. Prostate 1992;21:15–34.
8. Wei GL, Xiao SH, Liu CX. Pharmacokinetics of epristeride in rats and beagle dogs. Yao Xue Xue Bao 2000;35:721–4.
9. Benincosa LJ, Audet PR, Lundberg D, Zariffa N, Jorkasky DK. Pharmacokinetics and absolute bioavailability of epristeride in healthy male subjects. Biopharm Drug Dispos 1996;17:249–58.
10. Levy MA, Brandt M, Sheedy KM, et al. Cloning, expression and functional characterization of type 1 and type 2 steroid 5α-reductases from cynomolgus monkey: comparisons with human and rat isoenzymes. J Steroid Biochem Mol Biol 1995;52:307–19.
11. McNulty AM, Audia JE, Bemis KG, Goode RL, Rocco VP, Neubauer BL. Kinetic analysis of LY320236: competitive inhibitor of type I and non-competitive inhibitor of type II human steroid 5α-reductase. J Steroid Biochem Mol Biol 2000;72:13–21.
12. Carlin JR, Hoglund P, Eriksson LO, et al. Disposition and pharmacokinetics of [^{14}C]finasteride after oral administration in humans. Drug Metab Dispos 1992;20:148–55.
13. Ohtawa M, Morikawa H, Shimazaki J. Pharmacokinetics and biochemical efficacy after single and multiple oral administration of *N*-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, a new type of specific competitive inhibitor of testosterone 5α-reductase, in volunteers. Eur J Drug Metab Pharmacokinet 1991;16:15–21.
14. Tian G, Mook RA, Jr., Moss ML, Frye SV. Mechanism of time-dependent inhibition of 5α-reductases by Δ1-4-azasteroids: toward perfection of rates of time-dependent inhibition by using ligand-binding energies. Biochemistry 1995;34:13453–9.
15. Gisleskog PO, Hermann D, Hammarlund-Udenaes M, Karlsson MO. The pharmacokinetic modelling of GI198745 (dutasteride), a compound with parallel linear and nonlinear elimination. Br J Clin Pharmacol 1999;47:53–8.
16. Ju XB, Wu HF, Hua LX, Zhang W, Li J. The clinical efficacy of epristeride in the treatment of benign prostatic hyperplasia. Zhonghua Nan Ke Xue 2002;8:42–4.
17. Hudson PB, Boake R, Trachtenberg J, et al. Efficacy of finasteride is maintained in patients with benign prostatic hyperplasia treated for 5 years. The North American Finasteride Study Group. Urology 1999;53:690–5.
18. McConnell JD, Wilson JD, George FW, Geller J, Pappas F, Stoner E. Finasteride, an inhibitor of 5α-reductase, suppresses prostatic dihydrotestosterone in men with benign prostatic hyperplasia. J Clin Endocrinol Metab 1992;74:505–8.
19. Debruyne F, Barkin J, van Erps P, Reis M, Tammela TL, Roehrborn C. Efficacy and safety of long-term treatment with the dual 5α-reductase inhibitor dutasteride in men with symptomatic benign prostatic hyperplasia. Eur Urol 2004;46:488–94; discussion 495.
20. Clark RV, Hermann DJ, Cunningham GR, Wilson TH, Morrill BB, Hobbs S. Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5α-reductase inhibitor. J Clin Endocrinol Metab 2004;89:2179–84.
21. Presti JC, Jr., Fair WR, Andriole G, et al. Multicenter, randomized, double-blind, placebo controlled study to investigate the effect of finasteride (MK-906) on stage D prostate cancer. J Urol 1992;148:1201–4.
22. Andriole G, Lieber M, Smith J, et al. Treatment with finasteride following radical prostatectomy for prostate cancer. Urology 1995;45:491–7.
23. Stuart JD, Frank WL, Noel DS, et al. Pharmacokinetic parameters and mechanisms of inhibition of rat type 1 and 2 steroid 5α-reductases: determinants for different *in vivo* activities of GI198745 and finasteride in the rat. Biochem Pharmacol 2001;62:933–42.
24. Tian G. *In vivo* time-dependent inhibition of human steroid 5α-reductase by finasteride. J Pharm Sci 1996;85:106–11.
25. Ishii Y, Mukyama H, Ohtawa M. *In vitro* biotransformation of finasteride in rat hepatic microsome: isolation and characterization of metabolites. Drug Metab Dispos 1994;22:79–84.
26. Brendler CB, Follansbee AL, Isaacs JT. Discrimination between normal, hyperplastic and malignant human prostatic tissues by enzymatic profiles. J Urol 1985;133:495–501.
27. Bonkhoff H, Stein U, Aumuller G, Remberger K. Differential expression of 5α-reductase isoenzymes in the human prostate and prostatic carcinomas. Prostate 1996;29:261–7.
28. Luo J, Dunn TA, Ewing CM, Walsh PC, Isaacs WB. Decreased gene expression of steroid 5α-reductase 2 in human prostate cancer: implications for finasteride therapy of prostate carcinoma. Prostate 2003;57:134–9.
29. Thomas LN, Lazier CB, Gupta R, et al. Differential alterations in 5α-reductase type 1 and type 2 levels during development and progression of prostate cancer. Prostate 2005;63:231–9.
30. Titus MA, Gregory CW, Ford OH III, Schell MJ,

Maygarden SJ, Mohler JL. Steroid 5α-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res 2005;11:4365–71.

31. Deslypere JP, Young M, Wilson JD, McPhaul MJ. Testosterone and 5-αdihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTA-CAT reporter gene. Mol Cell Endocrinol 1992;88:15–22.

32. Brooks JR, Berman C, Nguyen H, et al. Effect of castration, DES, flutamide and the 5α-reductase inhibitor, MK906, on the growth of the Dunning rat prostatic carcinoma, R-3327. Prostate 1991;18:215–27.

33. Isaacs JT, Isaacs WB, Feitz WFJ, Scheres J. Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancers. Prostate 1986;9:261–81.

34. van Bokhoven A, Varella-Garcia M, Korch C, et al. Molecular characterization of human prostate carcinoma cell lines. Prostate 2003;57:205–25.

35. Dalrymple S, Antony L, Xu Y, et al. Role of notch-1 and E-cadherin in the differential response to calcium in culturing normal versus malignant prostate cells. Cancer Res 2005;65:9269–79.

36. Azzolina B, Ellsworth K, Andersson S, Geissler W, Bull HG, Harris GS. Inhibition of rat α-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J Steroid Biochem Mol Biol 1997;61:55–64.

37. Soderstrom TG, Bjelfman C, Brekkan E, et al. Messenger ribonucleic acid levels of steroid 5α-reductase 2 in human prostate predict the enzyme activity. J Clin Endocrinol Metab 2001;86:855–8.

38. Shirakawa T, Okada H, Acharya B, et al. Messenger RNA levels and enzyme activities of 5α-reductase types 1 and 2 in human benign prostatic hyperplasia (BPH) tissue. Prostate 2004;58:33–40.

39. Smith CM, Ballard SA, Worman N, Buettner R, Masters JRW. 5α-Reductase expression by prostate cancer cell lines and benign prostatic hyperplasia *in vitro*. J Clin Endocrinol Metab 1996;81:1361–6.

40. Bramson HN, Hermann D, Batchelor KW, Lee FW, James MK, Frye SV. Unique preclinical characteristics of GG745, a potent dual inhibitor of 5AR. J Pharmacol Exp Ther 1997;282:1496–502.

41. Lazier CB, Thomas LN, Douglas RC, Vessey JP, Rittmaster RS. Dutasteride, the dual 5α-reductase inhibitor, inhibits androgen action and promotes cell death in the LNCaP prostate cancer cell line. Prostate 2004;58:130–44.

42. Gao J, Arnold JT, Isaacs JT. Conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth during malignant transformation of prostatic epithelial cells. Cancer Res 2001;61:5038–44.

43. Ornstein DK, Smith DS, Andriole GL. Biochemical response to testicular androgen ablation among patients with prostate cancer for whom flutamide and/or finasteride therapy failed. Urology 1998;52:1094–7.

44. Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res 2005;11:4653–7.

45. Singh P, Uzgare A, Litvinov I, Isaacs JT. Combinatorial androgen receptor targeted (i.e., CART) therapy for prostate cancer. Endocr Relat Cancer. In press 2006.