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Advances in Brief

Selection for Androgen Receptor Mutations in Prostate Cancers Treated with Androgen Antagonist[1]

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Abstract

The role of androgen receptor (AR) mutations in androgen-independent prostate cancer (PCa) was determined by examining AR transcripts and genes from a large series of bone marrow metastases. Mutations were found in 5 of 16 patients who received combined androgen blockade with the AR antagonist flutamide, and these mutant ARs were strongly stimulated by flutamide. In contrast, the single mutant AR found among 17 patients treated with androgen ablation monotherapy was not flutamide stimulated. Patients with flutamide-stimulated AR mutations responded to subsequent treatment with bicalutamide, an AR antagonist that blocks the mutant ARs. These findings demonstrate that AR mutations occur in response to strong selective pressure from flutamide treatment.

Introduction

PCa[3] is dependent upon androgen stimulation mediated by the AR, a member of the steroid hormone receptor family of ligand-dependent nuclear receptors. The primary treatment for metastatic PCa is removal of testicular androgens, carried out most often by surgical or medical castration (androgen ablation monotherapy). Many patients are also treated with an AR antagonist (antiandrogen) to block the effects of androgens produced by the adrenal glands (commonly referred to as combined androgen blockade). Although most patients initially respond, all eventually relapse with tumors that are clinically androgen independent (1). The mechanisms through which these tumors develop androgen independence are not known and may be diverse. Mutations in the AR and amplification of the *AR* gene have been identified in some androgen-independent prostate cancers (2–7), but how and to what extent these changes contribute to the development of androgen-independent PCa remain unclear. This study determined the role of AR mutations in androgen-independent PCa through an analysis of metastatic androgen-independent prostate cancers in bone marrow biopsies from a large series of patients.

Materials and Methods

Tissue Acquisition. After informed consent, a bone marrow biopsy was obtained from the posterior iliac crest in patients with androgen-independent PCa, as described previously (5). Frozen sections were then analyzed histologically for tumor. PCa was identified in the bone marrow biopsies from

~40% of the patients biopsied, and these samples form the basis of this analysis.

RT-PCR Analysis of AR Transcripts. RNA was extracted from approximately five adjacent frozen sections of 6 μm using RNazol-B (TM Cinna Scientific, Friendswood, TX), and cDNA was synthesized with MMTV reverse transcriptase and an AR antisense primer located in the 3′ UTR (GCAAATAGAATTCAGGAACA). PCR amplifications were carried out with Taq DNA polymerase, a sense primer in the ligand binding domain (ACTCCTTTGCAGCCTTGC), and an internal antisense primer in the 3′ UTR (ACAGACTGTACATCAATAGAGGAAATTC) for 25 cycles. Secondary PCR amplifications were carried out for 25 cycles with nested 5′ (GCTCTAGACTCAATGAACTGGGAGAGAGAC) and 3′ UTR (GGCACTGCAGAGGAGTAGTGCAGA) primers, the former introducing an *Xba*I site. Negative control samples in which the reverse transcriptase step was omitted were included in all amplifications and did not amplify. The PCR products were cloned, and at least five colonies were sequenced. The region sequenced in all clones was from codon 701 through the stop codon. For direct sequencing of uncloned PCR products, the products were purified using a Microcon-100 membrane (Amicon, Beverly, MA).

Genomic DNA Sequencing. DNA was extracted from four to five adjacent frozen sections by overnight proteinase K digestion, followed by phenol/chloroform extraction. The DNA was PCR amplified using a primer in intron 7 (GAGGCCACCTCCTTGTCAACCCTG) and primers in the 3′ UTR of exon 8. The PCR products were purified and directly sequenced as above.

Transient Transfections. CV-1 cells in 24-well plates were transfected using Lipofectamine according to the manufacturer's directions (Life Technologies, Inc.). The wild-type AR expression vector, pSV.ARo, was kindly provided by Dr. A. Brinkmann (Erasmus University, Rotterdam, the Netherlands), and mutant ARs were constructed from this as described (5). The reported plasmid, pMMTVpLuc, was provided by Dr. Richard Pestell (Albert Einstein, New York, NY) and was also described previously (5). Each transfection was normalized for β-galactosidase activity from the cotransfected plasmid pSVgal (Promega Corp., Madison, WI). Each well received 20 ng of AR expression vector, 200 ng of pMMTVLuc, and 50 ng of pSVgal. Transfected cells were assayed after 24 h in medium containing charcoal/dextran-stripped FCS (Hyclone, Logan, UT) and the indicated hormone or AR antagonist. All samples were done in quadruplicate, and data shown are representative of at least three experiments.

Results

AR Mutations Detected by RT-PCR. Bone marrow is the most frequent site of metastatic PCa and can provide a source of tumor cells uncontaminated with benign prostatic tissue, which also expresses the AR. It was shown previously that the *AR* gene, which is on the X chromosome, was expressed at very low or undetectable levels in bone marrow biopsies that did not contain histological evidence of tumor (5). Therefore, RT-PCR was used to amplify the ligand binding domain of AR transcripts derived from tumor cells in histologically PCa-positive biopsies. The PCR products were cloned, and multiple clones (at least five per biopsy) were analyzed by DNA sequencing. All mutations were confirmed by repeating the PCR amplification,

cloning, and sequencing of AR transcripts from another aliquot of cDNA.

In the group of 17 evaluable patients treated initially by androgen ablation without an AR antagonist, a single patient with a point mutation was identified (patient 51, aspartate to asparagine in codon 890; Table 1). As shown by direct sequencing of AR PCR products, this mutation was present in the majority of AR transcripts (Fig. 1*A*). Analysis of genomic DNA from peripheral blood lymphocytes confirmed that the D890N change in this patient was a mutation and not a polymorphism (data not shown).

In contrast, AR mutations were detected in 5 of the 16 evaluable patients who received combined androgen blockade with the AR antagonist flutamide (Table 1). In all five cases, a mutation in codon 877, ACT (threonine) to GCT (alanine; T877A) was found. In four of these patients, the T877A was the only mutant AR isolated and was found in most or all of the clones sequenced. A second codon 877 mutation, ACT (threonine) to AGT (serine), was identified in one patient (Table 1, patient 14) and represented the majority of clones isolated from this patient. Direct sequencing of uncloned PCR-amplified AR transcripts from patients 2, 6, 12, and 55 confirmed that the T877A mutant represented most of the AR transcripts (Fig. 1*A*). Direct sequencing similarly confirmed that the T877S mutant was the major transcript in patient 14.

AR Mutations Detected by Analysis of Genomic DNA. In four of the patients with mutant ARs, the initial frozen sections contained predominantly tumor (not shown). Therefore, genomic DNA from these biopsies could be readily analyzed to further confirm the RT-PCR-based results and to determine whether the AR mutations were in a large fraction of the tumor cells *versus* a small fraction expressing high levels of AR message. Further frozen sections were cut from the appropriate blocks and again analyzed histologically, confirming that the sections were largely replaced by tumor (Fig. 1*B*). DNA was then extracted from adjacent frozen sections, and a portion of exon 8, containing codons 877 and 890, was amplified with a 5′ primer in intron 7 and 3′ exon primers and directly sequenced.

The D890N mutation was clearly observed in the DNA from patient 51 (Fig. 1*C*), demonstrating that this mutation was present in most or all of the tumor cells. The sequence analysis of genomic DNA from patients 6 and 12 similarly demonstrated that most or all of the tumor cells from these biopsies had the T877A mutant AR (Fig. 1*C*). In patient 14, only a minority of the PCR-amplified genomic DNA had the T877S mutation (AGT to ACT on the antisense strand in Fig. 1*C*). A larger fraction had the T877A mutation (AGT to AGC on the antisense strand), indicating that tumor cells with the former mutation expressed higher levels of AR transcripts.

Functional Analysis of Mutant ARs. Mutations in codon 877 were identified previously in advanced PCa from patients (3–6) and in a PCa cell line, LNCaP (8). Importantly, functional studies have shown that both the T877A and T877S mutations alter the AR so that hydroxyflutamide, the active metabolite of the AR antagonist flutamide, becomes a strong agonist (8–10). Therefore, identification here of these codon 877 mutations in patients who were treated with flutamide suggested that AR mutations resulting in activation by flutamide were positively selected by the drug. The D890N mutation

Table 1 *Androgen receptors in androgen-independent PCa patients*

	Primary treatment[a]	Response (mo)	Flutamide withdrawal response	AR	PSA decline in response to bicalutamide
1	LHRH	15		wt	None
2	LHRH+F	20	None	Codon 877 ACT(Thr)→GCT(Ala)	86%[b]
4	ORCH+F	36	None	wt	10%
5	LHRH+F	12	None	wt	25%
6	LHRH+F	24	None	Codon 877 ACT(Thr)→GCT(Ala)	30%
9	LHRH[c]	24		wt	None
12	LHRH+F	24	12 mo	Codon 877 ACT(Thr)→GCT(Ala)	>99%[d]
13	LHRH+F	48	None	wt	None
14	LHRH+F	20	None	Codon 877 ACT(Thr)→AGT(Ser) Codon 877 ACT(Thr)→GCT(Ala)	51%
16	ORCH[c]	12		wt	None
19	ORCH	18		No amplification	
20	LHRH+F	2	None	wt	None
22	LHRH	42		No amplification	
24	ORCH	12		No amplification	
41	ORCH[e]	17		wt	
42	ORCH+F	9	None	No amplification	
43	DES[f]	72		No amplification	
44	ORCH	14		wt	
45	ORCH	NA		wt	
46	ORCH[g]	19		wt	
47	LHRH[c]	20		wt	
48	LHRH+F	20	None	wt	
49	LHRH	15		wt	
50	LHRH	24		No amplification	
51	LHRH	12		Codon 890 GAC(Asp)→AAC(Asn)	None
52	LHRH	18		wt	None
53	LHRH+F	6	NA[h]	wt	
54	LHRH	38		wt	
55	LHRH+F	32	NA	Codon 877 ACT(Thr)→GCT(Ala)	
57	LHRH+F	3	NA	wt	
59	LHRH+F	13	NA	wt	
60	LHRH+F	12	NA	wt	
63	LHRH+F	33	NA	wt	

[a] Treatments were LHRH agonist (LHRH), orchiectomy (ORCH), LHRH agonist plus flutamide (LHRH+F), or diethylstilbestrol (DES). wt, wild type.

[b] Reflects PSA decline after 4 months of therapy, at which time bicalutamide was discontinued because of a possible drug interaction.

[c] No response to subsequent flutamide treatment.

[d] PSA fell to undetectable during therapy.

[e] Fifteen-month response to subsequent flutamide; no flutamide withdrawal response.

[f] Thirty-month response to subsequent orchiectomy plus flutamide; positive flutamide withdrawal response.

[g] Twelve-month response to subsequent flutamide; no flutamide withdrawal response.

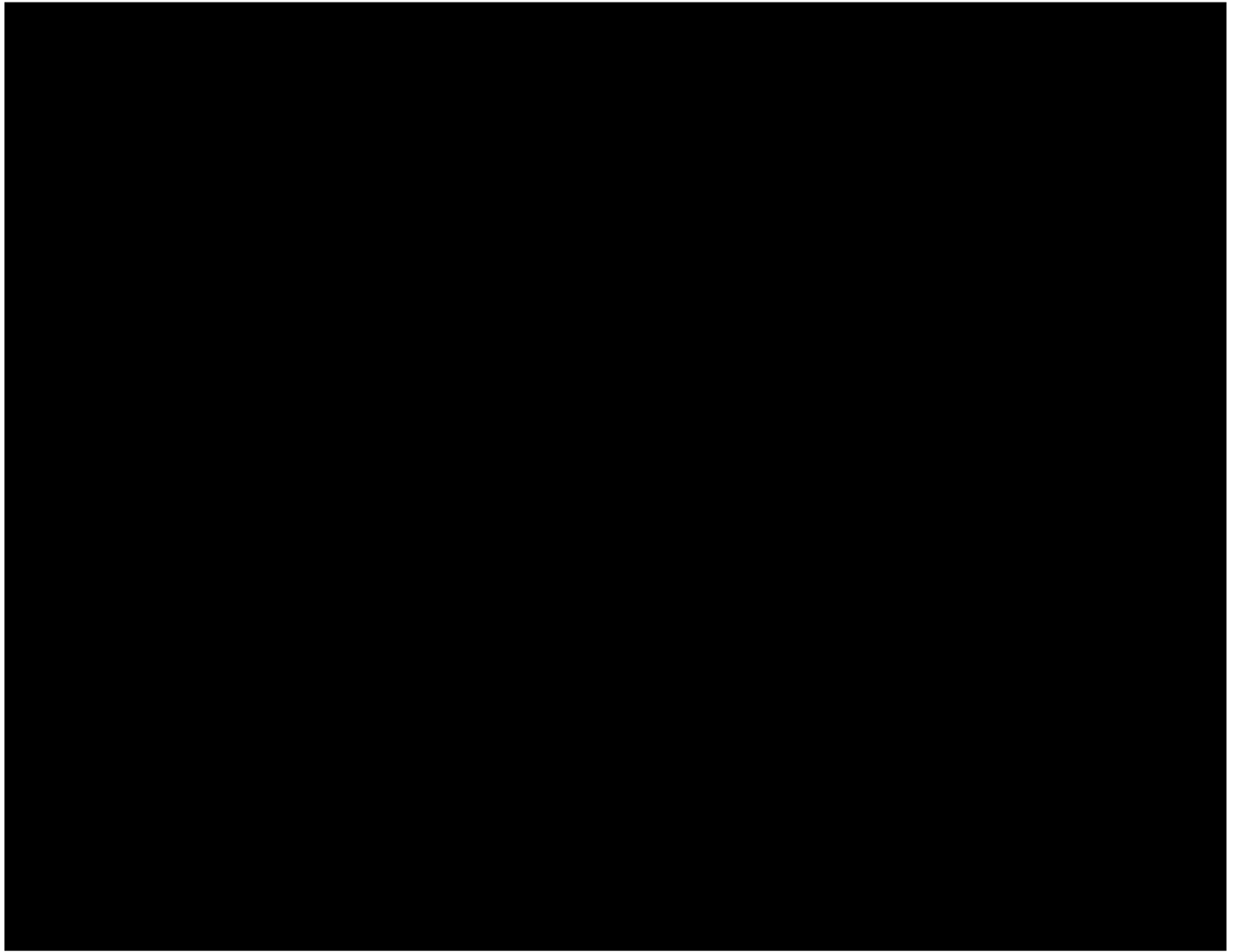[h] Data on these patients' responses were not available.

Fig. 1. Direct sequencing of PCR-amplified AR transcripts and genes. *A*, direct sequencing of RT-PCR products. The region containing the mutation is shown from the positive strand for patient 51 and negative strand for the remaining patients. The wild-type sequence is shown below each panel, and the amino acid encoded by the mutant AR is shown above. *B*, representative regions of sections used for DNA extraction from the indicated patients. *C*, direct sequencing of extracted and amplified genomic DNA. The negative strand sequence is shown for each, and the wild-type sequences for codons 890 (patient 51) and 877 (patients 6, 12, and 14) are shown.

was found in a patient who was not treated with flutamide. This mutant AR was analyzed functionally for its response to hydroxyflutamide in comparison with the wild-type and T877A mutant ARs.

CV-1 cells were transiently transfected with wild-type or mutant AR expression vectors and a luciferase reporter gene regulated by the androgen-responsive elements in the MMTV-LTR, pMMTVLuc (5, 10). The wild-type and mutant ARs showed minimal activity in steroid hormone-free medium but were strongly stimulated ($\sim$100-fold induction) by DHT (Fig. 2). As shown previously, the T877A (Fig. 2) and T877S (not shown) mutant ARs were strongly stimulated by hydroxyflutamide at concentrations <100 nM (10). This is likely significant *in vivo* because the serum concentrations achieved in patients are in the micromolar range (11). In contrast, the D890N AR was only weakly stimulated at the highest hydroxyflutamide concentration. Therefore, although the functional significance of the D890N mutation is not clear, the results support the conclusion that flutamide treatment selects for AR mutations that are strongly activated by hydroxyflutamide.

Clinical Responses to Secondary Hormonal Therapies. Some patients treated by androgen ablation in conjunction with flutamide

who subsequently develop androgen-independent PCa respond to discontinuation of their flutamide, a phenomena termed the flutamide withdrawal response (12, 13). Although flutamide-activated mutant ARs would be expected to result in flutamide withdrawal responses, this response was seen in only one of the five patients with a codon 877 mutation (patient 12, Table 1). An explanation for the lack of a flutamide withdrawal response in these patients is that these mutant ARs would have had ongoing activation by other steroid hormones, particularly those produced by the adrenals, when flutamide treatment was discontinued (10). Indeed, suppression of adrenal steroid hormone synthesis with aminoglutethimide has been reported to increase the response to flutamide withdrawal (14–16).

Both the T877A and T877S mutant ARs are inhibited by bicalutamide, a related nonsteroidal AR antagonist approved recently for prostate cancer treatment (10). Therefore, responses to this drug should be demonstrable *in vivo* in prostate cancer patients with these mutant ARs if they contribute significantly to the disease. Some of the patients studied here were enrolled in a clinical trial of high-dose bicalutamide (150 mg/day) immediately after bone marrow biopsy (17). Each of the patients with codon 877 mutations
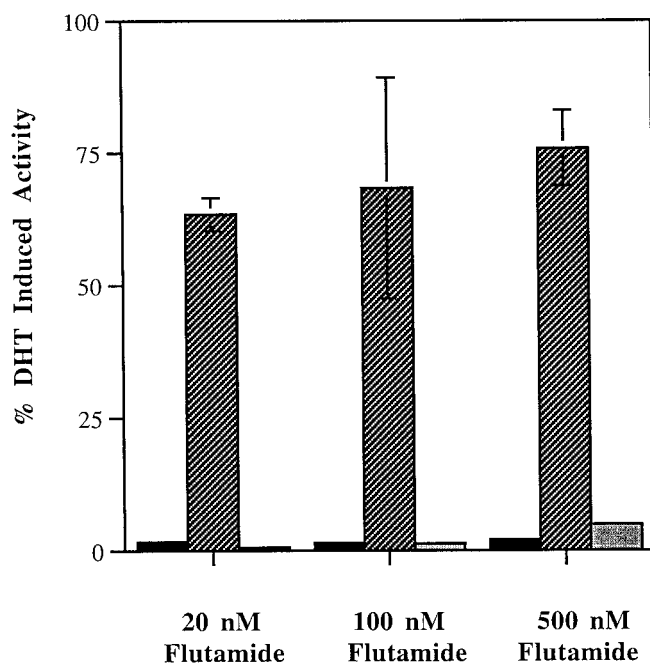
Fig. 2. Flutamide activation of mutant ARs. Transfected CV-1 cells were stimulated with DHT (100 nM) or hydroxyflutamide at 20, 100, or 500 nM (flutamide). Luciferase activity was determined, corrected for β-galactosidase activity, and expressed as a percentage of the maximal DHT-induced activity. All points were done in quadruplicate, and the results are the average; *bars,* ±1 SD. ■, wildtype; ▨, T877A; □, D890N.

enrolled had responses to the bicalutamide treatment, with their serum PSA levels declining by between 30% and > 99% (Table 1). These results indicated that ARs inhibited by bicalutamide, but not flutamide, contributed to tumor progression in these patients. The variability in responses to bicalutamide likely reflects tumor cell heterogeneity in these patients with advanced disease, which would clearly limit the clinical effects from this and other therapies.

Clinical data from this and another bicalutamide trial (17, 18) showed a significant correlation between PSA responses, clinical responses, and previous flutamide treatment. It is not clear whether this reflects AR mutations in all cases, because responses to bicalutamide were seen in some flutamide-treated patients with wild-type ARs (Table 1, patients 4 and 5). Because the AR analysis in all patients was based upon a biopsy from a single site, tumor cells at other sites with AR mutations could have contributed to bicalutamide responses in some patients. Alternatively, because hydroxyflutamide, but not bicalutamide, is a weak agonist of the wild-type AR (10), mechanisms other that AR mutation may contribute to bicalutamide responses in patients treated previously with flutamide.

Discussion

These findings provide direct evidence that AR mutations occur in response to selective pressure from AR antagonist treatment and that these mutations contribute to PCa progression after androgen ablation therapy. Most significantly, the selection for rare tumor cells with hydroxyflutamide-stimulated mutant ARs indicates that ongoing AR activity is critical for tumor cell survival after androgen ablation and that mutation of the AR is an important mechanism for achieving this activity in flutamide-treated patients. In contrast, AR mutation appears to be a less important mechanism for maintaining AR stimulation in patients treated with androgen ablation monotherapy, perhaps due to the ability of residual adrenal androgens to provide an adequate

level of stimulation through the wild-type AR. Although the use of an AR antagonist appears to significantly alter the mechanisms used by tumor cells to escape from androgen ablation therapy, results from a series of clinical trials comparing androgen ablation monotherapy to combination therapy with flutamide or other AR antagonists have not shown substantial increases in progression-free or overall survival in the combination therapy groups, except perhaps in patients with low-volume disease (19, 20).

The hydroxyflutamide-activated mutant ARs identified here were all altered in codon 877. In a previous study, we identified two additional flutamide-treated patients with hydroxyflutamide-activated ARs, one with a codon 877 mutation (T877S) and one with a codon 874 mutation (H874Y; Refs. 5 and 10). The finding of particular codon 877 mutations in these and other studies (4, 6) suggests that this functional change can be mediated by only a very limited number of structural changes in the AR. It should also be noted that codon 874 and 877 mutations may provide selective advantages in addition to altering flutamide responses, because both have been identified in cell lines derived from patients not treated with flutamide (CWR22 and LNCaP cells, respectively; Refs. 8 and 21). A more weakly hydroxyflutamide-activated mutant AR (V715M) was also identified previously in a patient treated with flutamide (2, 10).

These results have several significant implications for the hormonal therapy of PCa: (*a*) the selection for rare tumor cells with mutant ARs indicates that AR antagonists may improve responses to androgen ablation therapy initially but subsequently accelerate the growth of surviving tumor cells; (*b*) alternative methods to further block the AR-mediated signaling that appears critical for tumor cell survival, such as targeting AR-associated coactivator proteins, downstream target genes, or signal transduction pathways that interact with the AR, may enhance responses to androgen ablation monotherapy or combined androgen blockade; and (*c*) the additive or synergistic effects of cytotoxic chemotherapy used early in conjunction with androgen ablation therapy may be very effective if, as this study suggests, only a relatively small number of tumor cells survive combined androgen blockade.

Acknowledgments

References

1. Catalona, W. J. Management of cancer of the prostate [see comments]. N. Engl. J. Med., *331:* 996–1004, 1994.
2. Culig, Z., Hobisch, A., Cronauer, M. V., Cato, A. C., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G., and Klocker, H. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. Mol. Endocrinol., *7:* 1541–1550, 1993.
3. Gaddipati, J. P., McLeod, D. G., Heidenberg, H. B., Sesterhenn, I. A., Finger, M. J., Moul, J. W., and Srivastava, S. Frequent detection of codon 877 mutation in the androgen receptor gene in advanced prostate cancers. Cancer Res., *54:* 2861–2864, 1994.
4. Suzuki, H., Sato, N., Watabe, Y., Masai, M., Seino, S., and Shimazaki, J. Androgen receptor gene mutations in human prostate cancer. J. Steroid Biochem. Mol. Biol., *46:* 759–765, 1993.
5. Taplin, M. E., Bubley, G. J., Shuster, T. D., Frantz, M. E., Spooner, A. E., Ogata, G. K., Keer, H. N., and Balk, S. P. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer [see comments]. N. Engl. J. Med., *332:* 1393–1398, 1995.
6. Suzuki, H., Akakura, K., Komiya, A., Aida, S., Akimoto, S., and Shimazaki, J. Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to antiandrogen withdrawal syndrome. Prostate, *29:* 153–158, 1996.
7. Visakorpi, T., Hyytinen, E., Koivisto, P., Tanner, M., Keinanen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J., and Kallioniemi, O. P. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat. Genet., *9:* 401–406, 1995.

8. Veldscholte, J., Ris-Stalpers, C., Kuiper, G. G., Jenster, G., Berrevoets, C., Claassen, E., van Rooij, H. C., Trapman, J., Brinkmann, A. O., and Mulder, E. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. Biochem. Biophys. Res. Commun., *173:* 534–540, 1990.

9. Veldscholte, J., Berrevoets, C. A., Brinkmann, A. O., Grootegoed, J. A., and Mulder, E. Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation. Biochemistry, *31:* 2393–2399, 1992.

10. Fenton, M. A., Shuster, T. D., Fertig, A. M., Taplin, M-E., Kolvenbag, G., Bubley, G. J., and Balk, S. P. Functional characterization of mutant androgen receptors from androgen-independent prostate cancer. Clin. Cancer. Res., *3:* 1383–1388, 1997.

11. Belanger, A., Giasson, M., Couture, J., Dupont, A., Cusan, L., and Labrie, F. Plasma levels of hydroxy-flutamide in patients with prostatic cancer receiving the combined hormonal therapy: an LHRH agonist and flutamide. Prostate, *12:* 79–84, 1988.

12. Scher, H. I., and Kelly, W. K. Flutamide withdrawal syndrome: its impact on clinical trials in hormone-refractory prostate cancer. J. Clin. Oncol., *11:* 1566–1572, 1993.

13. Small, E. J., and Srinivas, S. The antiandrogen withdrawal syndrome. Experience in a large cohort of unselected patients with advanced prostate cancer. Cancer (Phila.), *76:* 1428–1434, 1995.

14. Dupont, A., Gomez, J. L., Cusan, L., Koutsilieris, M., and Labrie, F. Response to flutamide withdrawal in advanced prostate cancer in progression under combination therapy. J. Urol., *150:* 908–913, 1993.

15. Sartor, O., Cooper, M., Weinberger, M., Headlee, D., Thibault, A., Tompkins, A., Steinberg, S., Figg, W. D., Linehan, W. M., and Myers, C. E. Surprising activity of flutamide withdrawal, when combined with aminoglutethimide, in treatment of "hormone-refractory" prostate cancer [published erratum appears in J. Natl. Cancer Inst., *86:* 463, 1994]. J. Natl. Cancer Inst., *86:* 222–227, 1994.

16. Small, E. J., Baron, A., and Bok, R. Simultaneous antiandrogen withdrawal and treatment with ketoconazole and hydrocortisone in patients with advanced prostate carcinoma. Cancer (Phila.), *80:* 1755–1759, 1997.

17. Joyce, R., Fenton, M. A., Rode, P., Constantine, M., Gaynes, L., Kolvenbag, G., DeWolf, W., Balk, S., Taplin, M. E., and Bubley, G. J. High dose bicalutamide for androgen independent prostate cancer: effect of prior hormonal therapy. J. Urol., *159:* 149–153, 1998.

18. Scher, H. I., Liebertz, C., Kelly, W. K., Mazumdar, M., Brett, C., Schwartz, L., Kolvenbag, G., Shapiro, L., and Schwartz, M. Bicalutamide for advanced prostate cancer: the natural *versus* treated history of disease. J. Clin. Oncol., *15:* 2928–2938, 1997.

19. Caubet, J. F., Tosteson, T. D., Dong, E. W., Naylon, E. M., Whiting, G. W., Ernstoff, M. S., and Ross, S. D. Maximum androgen blockade in advanced prostate cancer: a meta-analysis of published randomized controlled trials using nonsteroidal antiandrogens. Urology, *49:* 71–78, 1997.

20. Eisenberger, M. A., Blumenstein, B. A., Crawford, E. D., Miller, G., McLeod, D. G., Loehrer, P. J., Wilding, G., Sears, K., Culkin, D. J., Thompson, I. M., Jr., Bueschen, A. J., and Lowe, B. A. Bilateral orchiectomy with or without flutamide for metastatic prostate cancer. N. Engl. J. Med., *339:* 1036–1042, 1998.

21. Tan, J., Sharief, Y., Hamil, K. G., Gregory, C. W., Zang, D. Y., Sar, M., Gumerlock, P. H., deVere White, R. W., Pretlow, T. G., Harris, S. E, Wilson, E. M., Mohler, J. L., and French, F. S. Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol. Endocrinol., *11:* 450–459, 1997.