Adrenal Androgen Levels as Predictors of Outcome in Prostate Cancer Patients Treated with Ketoconazole Plus Antiandrogen Withdrawal: Results from a Cancer and Leukemia Group B Study

Charles J. Ryan,[1] Susan Halabi,[2] San-San Ou,[2] Nicholas J. Vogelzang,[3] Philip Kantoff,[4] Eric J. Small,[1] and for the Cancer and Leukemia Group B

Abstract

Purpose: Adrenal androgens activate the androgen receptor and stimulate prostate cancer growth. Ketoconazole is used as an inhibitor of adrenal androgen synthesis in men with androgen-independent prostate cancer. This study analyzes the relationship between pretreatment androgen levels and outcome following ketoconazole treatment.

Experimental Design: Baseline levels of three adrenal androgens (androstenedione, dehydroepiandrostenedione, and dehydroepiandrostenedione-sulfate) and testosterone were measured. Regression models (logistic and proportional hazard) were used to assess the prognostic significance of these levels in predicting overall survival and prostate-specific antigen (PSA) response defined by Consensus Criteria.

Results: In 103 patients with available levels, PSA response rate was 28% and median response duration was 4.8 months. The median baseline androstenedione level was 0.64 ng/mL and was 0.88 ng/mL versus 0.53 ng/mL for those with and without a PSA response, respectively ($P = 0.034$). In univariate analysis, elevation of baseline androstenedione levels was predictive of PSA response [odds ratio, 2.26; 95% confidence interval (95% CI), 1.03-4.96; $P = 0.043$]. In multivariate analysis, both the uppermost and the middle tertile of baseline androstenedione level were associated with an improved overall survival compared with those in the lower tertile (hazard ratio, 0.59; 95% CI, 0.36-0.98; $P = 0.40$; hazard ratio, 0.53; 95% CI, 0.32-0.90; $P = 0.018$, respectively). A linear correlation was observed among all androgen levels.

Conclusions: Higher androstenedione levels predict likelihood of response to ketoconazole and improved survival compared with patients with lower levels. These data suggest that therapy with ketoconazole is less effective in patients with low levels of androgen at baseline.

The use of sequential hormonal therapies is a common practice in the systemic therapy of advanced prostate cancer. Patients who succumb to the disease typically do so only after all hormonal treatments have ceased to be effective, in what is referred to as androgen-independent prostate cancer (AIPC). The mechanisms leading to androgen-independent growth of prostate cancer are complex but are likely to involve continued androgen receptor (AR) signaling in the face of castrate levels of testosterone. A variety of mechanisms may be responsible for this phenomenon, including activation of the AR by adrenal androgens or exogenous growth factors, amplification of and mutations within the AR itself (1, 2), as well as alterations in the recruitment and activity of coactivating molecules (3). Further, it has been shown recently that AIPC cells express genes corresponding to the enzymes responsible for the conversion of adrenal androgens to testosterone (4). For those patients who experience disease progression despite therapy with an antiandrogen, the withdrawal of the antiandrogen has been reported to induce prostate-specific antigen (PSA) declines of $\geq$50% in $\sim$10% of patients (5, 6). This event, the antiandrogen withdrawal (AAWD) phenomenon, remains ill defined mechanistically but suggests that the antiandrogen itself may be stimulating the AR complex.

Although androgen deprivation therapy typically reduces serum testosterone levels by >95%, there is not a proportional reduction in adrenal androgens, such as androstenedione, dehydroepiandrostenedione, and dehydroepiandrostenedione-sulfate (DHEAS; ref. 7). The persistence of dehydroepiandrostenedione and androstenedione levels may have clinical

Authors' Affiliations: [1]University of California/San Francisco, San Francisco, California; [2]Cancer and Leukemia Group B Statistical Center, Duke University Medical Center, Durham, North Carolina; [3]University of Chicago Medical Center, Chicago, Illinois; and [4]Dana-Farber Cancer Institute, Harvard University, Boston, Massachusetts

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Note: Current address for N.J. Vogelzang: Nevada Cancer Institute, Las Vegas NV 89135.

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Requests for reprints: Charles J. Ryan, Urologic Oncology Program, University of California/San Francisco Comprehensive Cancer Center, 3rd Floor, 1600 Divisadero, San Francisco, CA 94143. Phone: 415-353-9279; Fax: 415-353-7779. E-mail: ryanc@medicine.ucsf.edu

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significance, as these androgens have been shown to activate both wild-type and mutant ARs *in vitro* (8, 9). Because adrenal androgens have been implicated in prostate cancer progression, clinical strategies targeting the production of adrenal androgens, such as adrenalectomy (10) and aminoglutethimide (11), have been used in the past. Contemporary studies have centered on the use of ketoconazole, an agent with oral bioavailability and a relatively favorable side effect profile (12, 13). Despite several studies that have shown activity of ketoconazole, little is known about the effects of ketoconazole on adrenal androgens or about the prognostic and predictive value of adrenal androgens levels at baseline in patients with AIPC.

Cancer and Leukemia Group B (CALGB) 9583 was a multicenter, randomized phase III clinical trial evaluating the clinical efficacy of ketoconazole when administered at the time of AAWD. In this study, 260 patients were randomized to either AAWD alone or AAWD plus ketoconazole (6). Adrenal androgen levels were prospectively measured in all patients, providing a unique data set with which to evaluate both the effects of ketoconazole on these levels. Further, these data provide an opportunity to determine the prognostic and predictive value of baseline and subsequent adrenal androgen levels and to test the hypothesis that androgen levels may help discriminate between those patients who are likely to benefit from treatment with ketoconazole from those who are not.

Materials and Methods

Patients. Eligible patients had histologically confirmed adenocarcinoma of the prostate with progressive metastatic disease and testosterone levels <50 ng/dL. All patients remained on a luteinizing hormone–releasing hormone agonist or were surgically castrated and had received at least 4 weeks of ongoing therapy with an antiandrogen. For patients with measurable disease, progression was defined as a >25% increase in the sum of the products of the perpendicular diameters of all measurable lesions. For patients with "bone only" disease, a PSA of >5 ng/mL, which had increased from baseline on at least two successive occasions at least 4 weeks apart, was required. Patients were excluded if they had received prior chemotherapy, immunotherapy, experimental therapy, or prior treatment with ketoconazole, aminoglutethemide, or corticosteroids. All participants signed an Institutional Review Board–approved, protocol-specific informed consent form in accordance with federal and institutional guidelines.

Treatment. After registration, patients were randomized with equal probability to AAWD alone (arm 1) or AAWD plus ketoconazole (arm 2) by the CALGB Statistical Center. Patients on arm 2 received 400 mg tid oral ketoconazole plus 40 mg/d oral hydrocortisone (30 mg a.m. and 10 mg p.m.) continuously until disease progression. Disease progression was defined as an increase in size of soft tissue lesions, new lesions on bone scan, or an increase in PSA by 50% over previous levels and a total change in PSA >5 ng/mL (confirmed by a second measurement 2 weeks apart), or unacceptable toxicity. Patients randomized to the AAWD alone arm were provided the option of crossover to treatment with ketoconazole on disease progression. The overall clinical results from this trial have been reported previously (6).

Eligible patients were evaluated with a history and physical examination at study entry and monthly thereafter. An endocrine panel (consisting of androstenedione, DHEAS, dehydroepiandrosterone, and testosterone levels) was obtained at baseline, at 1 and 3 months after starting therapy, and at the time of clinical disease progression. Blood samples were obtained between 8 and 10 a.m. Patient plasma samples were isolated, frozen, and shipped for analysis at a central commercial laboratory to determine androstenedione, DHEAS, dehydroepiandrostenedione, and testosterone levels using a liquid chromatography tandem mass spectrometry assay technique.

Statistical design and data analysis. The statistical design for the clinical end points of the trial has been described elsewhere (6). Accrual to the study began in October 1996 and was completed on May 2000. All data collection was managed by the CALGB Statistical Center. Patients were considered to have adrenal androgen (androstenedione, dehydroepiandrostenedione, and DHEAS) levels that were high (greater than the median) or low (below or equal the median). The study of the clinical significance of adrenal androgen levels in arm 1 was designed assuming that (*a*) there were 89 deaths, (*b*) the hazards in the two groups (adrenal androgen level below or equal the median or adrenal androgen level greater than median) are proportional, and (*c*) a two-sided type I error of 0.05, the log-rank statistic, would have 80% power to detect a hazard ratio (HR) for survival of 1.45 between patients whose androstenedione levels are high (dichotomized at greater than the median level) and low (below or equal the median).

Adrenal androgen levels were correlated with baseline PSA, alkaline phosphatase, performance status, overall survival, and PSA "response" (defined as a ≥50% decline in PSA from baseline using the PSA consensus criteria). Survival time was defined as the time from randomization to death. The Kaplan-Meier product limit estimator (14) was used to estimate the survival distribution by the two groups of androstenedione levels (based on the median androstenedione levels) and by the tertile values of androstenedione levels.

Further, the proportional hazards model (15) was used to assess the prognostic importance of androstenedione levels in predicting overall survival adjusting for other baseline predictors, such as PSA, alkaline phosphatase, lactate dehydrogenase (LDH), hemoglobin, and performance status.

In addition, statistical methods based on asymptotic distributions were used to find a cut point for androstenedione corresponding to the largest discrepancy between the lower- and higher-risk groups was based on the log-rank statistics. The exact P value was based on the maximally selected and was computed adjusting on multiple comparisons (16).

Logistic regression models were used to test whether adrenal androgens predict 50% decline in PSA from baseline. All of the tests were done using two-sided α level of 0.05.

Results

Patient characteristics

Two hundred sixty patients were randomized, 132 to arm 1 (AAWD alone) and 128 to arm 2 (AAWD plus ketoconazole). Patients were evenly matched between the arms with regards to a variety of prognostic and clinical features, including age, race, sites of metastatic disease, performance status, use of opioid analgesics, PSA, alkaline phosphatase, LDH, and serum creatinine (Table 1). Of 132 patients in arm 1, baseline adrenal androgen data are available from 113. Of 128 patients on arm 2, baseline and follow-up data are available from 103 patients.

The median age of patients in arm 2 was 71 (range, 49-90). Eighty-four percent had bone metastases, 31% had soft tissue metastases, and 13% had visceral metastases. The Eastern Cooperative Oncology Group performance status was 0 to 1 in 94%. The median pretreatment PSA was 58 ng/dL (range, 5-3,160). The median levels of alkaline phosphatase and hemoglobin were 124 (range, 40-880) and 12.6 (range, 7.4-15.2), respectively. Baseline and subsequent adrenal androgen

Table 1. Patient characteristics

	Baseline characteristics		
	AAWD (n = 132)	AAWD + Keto (n = 128)	Total (N = 260)
Age, y*	71 (66-76)	72 (64-76)	72 (65-76)
Race, % White	78	81	79
Sites of disease†			
Bone metastases	86%	84%	84%
Measurable disease	31%	39%	35%
Lymph node involvement	29%	35%	33%
Lung metastases	5%	6%	5%
Liver metastases	5%	10%	6%
Performance status (0-1)	93%	93%	93%
Opioid analgesic use	30%	28%	29%
Hemoglobin (g/dL)*	12.6 (11.7-13.3)	12.6 (11.1-13.6)	12.6 (11.6-13.5)
PSA (ng/mL)*	58 (17-162)	58 (20-137)	58 (20-151)
Alkaline phosphatase (IU/L)*	125 (91-239)	120 (85-225)	124 (90-235)
LDH (IU/L)*	200 (171-405)	215 (189-409)	210 (176-405)
Creatinine (mg/dL)*	1.0 (0.9-1.2)	1.1 (1.0-1.3)	1.1 (0.9-1.3)

*Median and interquartile range.
†Patients may have more than one metastasis.

levels are summarized in Table 2. The median baseline testosterone level was 13 ng/mL [interquartile ranges, 1-19]. The median baseline androstenedione, dehydroepiandrosterone, and DHEAS levels were 0.64 ng/mL (interquartile range, 0.5-1.1), 2.1 ng/mL (interquartile range, 1.6-3.3), and 318 ng/mL (interquartile range, 144-714), respectively. The median levels of androstenedione, dehydroepiandrostenedione, and DHEAS fell within the normal limits for adults. As reported previously, the levels of dehydroepiandrostenedione, DHEAS, and androstenedione declined after 1 month of therapy with ketoconazole (Table 2). At the time of clinical progression, the levels of DHEAS had increased by over 3-fold and androstenedione had increased by >50% when compared with the level after 1 month of therapy, both values associated with $P < 0.001$ (6).

Clinical outcomes

In arm 1 (AAWD alone), 13% [95% confidence interval (95% CI), 9-23%] of the patients experienced a 50% or greater decline in PSA and 2% (95% CI, 2-11%) experienced objective responses. The median survival was 16.7 months (95% CI, 14.3-21.5) and the median time to PSA progression was 2.1 months (95% CI, 1.4-2.9).

Of the 103 patients treated with ketoconazole in arm 2, 89 (86%) have died and the median follow-up time among surviving patients is 51.5 months. The median survival time among the 103 patients was 17.4 months (95% CI, 13.4-20.8). The median time to progression for patients in this cohort was 3.44 (interquartile range, 2.05-4.96) months. Twenty-nine (28%) patients experienced a 50% decline in PSA from baseline on treatment with ketoconazole, with a median duration of response of 4.8 months (interquartile range, 2.8-9.1; ref. 6).

Association of adrenal androgen levels with response to therapy

Arm 1 (AAWD alone). Baseline adrenal androgen data are available from 113 of the patients in this cohort. In univariate analysis of the relationship of all androgens, stratified at the median, to PSA decline during AAWD, there was a trend toward an inverse relationship between androstenedione levels and response (odds ratio, 0.33; 95% CI, 0.10-1.14; $P = 0.08$). The relationship of all other baseline adrenal androgen levels to PSA decline on AAWD was not significant. Stratification by androstenedione tertiles showed only a trend between the highest and the lowest baseline levels of androstenedione and likelihood of a 50% decline in PSA. The univariate HR for the middle tertile versus lower tertile was 0.69 (95% CI, 0.22-2.17; $P = 0.52$) and comparing the upper tertile versus the lower tertile was 0.14 (95% CI, 0.02-1.21; $P = 0.07$).

Arm 2 (AAWD plus ketoconazole). In univariate analysis of the relationship of each adrenal androgen measured with response to therapy, only baseline androstenedione levels predicted a 50% decline in PSA. Patients with high baseline androstenedione levels were more likely to experience a 50%

Table 2. Median adrenal androgen levels in AWD/ketoconazole-treated patients (interquartile) at baseline and after 1 mo on ketoconazole

	Baseline, N = 103	After 1 month
DHEA	2.1	1.0
Median (25%, 75%)	(1.6,3.3)	(0.8,1.4)
Range	0.6-9.3	75 (0.5-3.0)
DHEAS	318	30
Median (25%, 75%)	(144,714)	(1,77)
Range	1-1,770	76 (1-613)
Androstenedione	0.64	0.31
Median (25%, 75%)	(0.5,1.1)	(0.18,0.42)
Range	0.47-1.15	75 (0.10-3.36)
Testosterone	13	11
Median (25%, 75%)	(1,19)	(1,16)
Range	1-59	76 (1-39)

NOTE: Levels of dehydroepiandrostenedione, DHEAS, and androstenedione are within the reference range for adult males.
Abbreviations: Keto, ketoconazole; DHEA, dehydroepiandrostenedione.

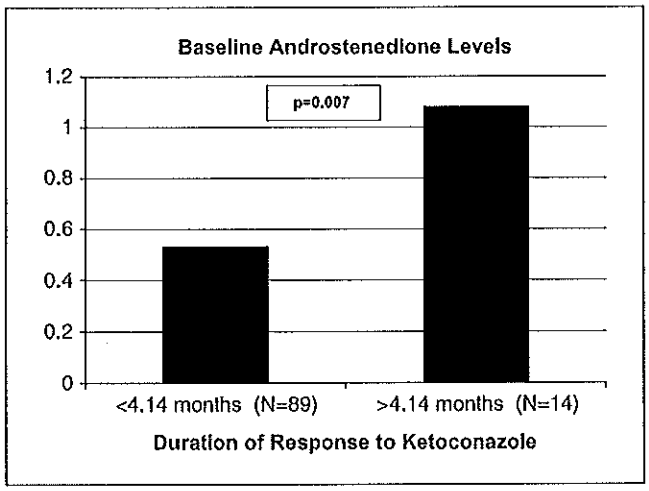

Fig. 1. Relationship between baseline androstenedione levels and duration of response to ketoconazole. The baseline androstenedione levels are stratified by the median duration of response to ketoconazole.

decline in PSA (odds ratio, 2.26; 95% CI, 1.03-4.96; $P = 0.043$). Baseline dehydroepiandrostenedione (odds ratio, 1.30; 95% CI, 0.94-1.78; $P = 0.11$) and DHEAS levels (odds ratio, 0.87; 95% CI, 0.48-1.56; $P = 0.64$) were not associated with PSA responses. The median androstenedione for patients with and without a 50% decline in PSA, respectively, was 0.88 ng/mL (95% CI, 0.52-1.30) versus 0.53 ng/mL (95% CI, 0.43-1.10; $P = 0.03$, Wilcoxon; Fig. 1).

In a multivariate analysis of the relationship between androstenedione levels and PSA response, which corrected for PSA, performance status, alkaline phosphatase, and hemoglobin at baseline, the significance of the relationship between PSA response and baseline androstenedione did not persist. The odds ratio (95% CI) for PSA response is divided by tertiles: middle versus lowest, 1.62 (0.54-4.92; $P = 0.393$); high versus the low, 2.25 (0.74-6.85; $P = 0.154$).

Association of adrenal androgen levels with survival

Because of the relationship of androstenedione levels to response to therapy with ketoconazole, the relationship between baseline androstenedione levels and survival was evaluated.

In arm 1 (AAWD alone), the median androstenedione level was 0.68, and the survival (interquartile) above and below the median value was 21.7 (16.7-28.5) and 15.1 (10.1-18.6) months, respectively, a difference that was not also statistically significant ($P = 0.23$).

In patients treated with immediate ketoconazole (arm 2), the median survival among patients whose baseline androstenedione levels were above the median value of 0.64 ng/mL was 19.7 months (95% CI, 13.5-29.5) compared with 14.3 months (95% CI, 10.9-19.2) in patients with baseline levels below the median, a difference that was not statistically significant ($P = 0.128$).

An exploratory analysis was done to determine whether patients with the lowest androstenedione levels had the worst prognosis, irrespective of the median value. This analysis was based on the hypothesis that tumor progression with low androgen levels would be less responsive to a therapy, such as ketoconazole. Baseline androstenedione levels are divided into low, intermediate, and high. The median survival for upper, middle, and lower androstenedione tertiles were 15.7 months (95% CI, 10.9-26.7), 21.2 months (95% CI, 14.7-43.9), and 13.9 months (95% CI, 9.1-19.2), respectively ($P_{trend} = 0.021$). In multivariate analysis of outcome, where the patients in arm 2 were treated with immediate ketoconazole, which corrected for PSA, alkaline phosphatase, performance status, and hemoglobin, the HR for death was 0.53 (95% CI, 0.32-0.90; $P = 0.018$) comparing the values for the middle versus the lower tertile and was 0.59 (95% CI, 0.36-0.98) comparing the highest tertile to the lowest ($P = 0.04$). In arm 1 (AAWD alone), the HR for survival of the middle versus lower tertile was 1.29 (95% CI, 0.80-2.09; $P = 0.29$) and comparing the upper versus lower tertile was 1.65 (95% CI, 0.95-2.85; $P = 0.08$), reflecting a trend toward statistical significance.

An exploratory multivariate analysis (Table 3) was done to test the association of baseline androstenedione levels to overall survival of all 197 patients who received ketoconazole on the study (103 at the time of randomization and 94 who crossed over to ketoconazole therapy following progression after AAWD) to laboratory variables that are of prognostic significance in patients with advanced prostate cancer (PSA, performance status, alkaline phosphatase, LDH, and hemoglobin; ref. 15) and to correct for whether therapy with ketoconazole occurred at the time of randomization or at crossover. In this analysis, an improved overall survival was observed for patients in the middle androstenedione tertile compared with those in the lower tertile (HR, 0.69; 95% CI, 0.47-0.99; $P = 0.04$), whereas no such association existed comparing those in the upper tertile to the lowest tertile 0.94 (95% CI, 0.66-1.33; $P = 0.72$).

To determine what variables may confound these data, a variety of clinical factors and levels of other androgens were evaluated according to their relationship to androstenedione, divided by tertiles of low, medium, and high. A stepwise correlation among all androgen levels was observed: dehydroepiandrostenedione, DHEAS, and testosterone levels were higher in the group of patients in the middle and higher androstenedione tertile than in the lowest tertile ($P < 0.001$,

Table 3. Multivariate model of baseline Androstenedione levels (tertile) predicting overall survival in all patients adjusted for the time of ketoconazole treatment ($N = 197$)

Factor	HR (95% CI)	P
Androstenedione		
Middle tertile vs low	0.69 (0.47-0.99)	0.044
High tertile vs. low	0.94 (0.66-1.33)	0.720
PSA (>54 vs ≤54)	1.81 (1.31-2.50)	<0.001
Performance status (2 vs 0,1)	1.06 (0.72-1.57)	0.752
Alkaline phosphatase (>124.5 vs ≤124.5)	1.40 (1.03-1.91)	0.035
Hemoglobin (>12.6 vs ≤12.6)	0.91 (0.67-1.22)	0.518
Rx of keto at randomization vs at crossover	1.12 (0.78-1.61)	0.533

Abbreviation: Rx, treatment.

Table 4. Baseline variables correlated to baseline androstenedione levels

	Androstenedione				
	≤0.50 (n = 37)	0.51-0.97 (n = 32)	>0.97 (n = 34)	Total (N = 103)	P
Age	73 (69-78)	71 (63-77)	70 (61-74)	72 (62-76)	0.16
LDH	235 (198-542)	230 (190-404)	196 (152-236)	212 (190-443)	0.01
Hemoglobin	12.4 (10.9-13.3)	13.2 (11.9-13.9)	12.3 (11.1-13.4)	12.6 (11.2-13.6)	0.14
PSA	55 (15-156)	45.5 (15.0-73.5)	93 (24-243)	58 (20-156)	0.07
Alkaline phosphatase	130 (95-244)	121 (90-189)	119 (82-275)	125 (90-226)	0.81
DHEA	1.9 (1.2-2.1)	2.1 (1.6-2.8)	3.3 (2.3-3.9)	2.1 (1.6-3.3)	<0.001
DHEAS	206 (89-414)	258 (164-659)	529 (164-1039)	318 (144-714)	0.01
Testosterone	10 (1-12)	13 (1-18)	19 (13-24)	13 (1-19)	<0.001
Gleason sum					
2-4	1 (3)	3 (10)	0 (0)	4 (4)	
5-7	17 (50)	14 (45)	17 (52)	48 (49)	0.53
8-10	16 (47)	14 (45)	16 (48)	46 (47)	
Performance status					
1	19 (53)	13 (40)	11 (32)	43 (42)	
2	17 (47)	19 (59)	23 (68)	59 (58)	0.23

P = 0.01, and P < 0.001, respectively). A significant trend in LDH levels opposite that of androstenedione was observed: the lowest androstenedione tertile had the highest LDH levels. No other variables were statistically significant and none of the findings readily explain the fact that patients in the middle androstenedione tertile had the best survival. These results are shown in Table 4.

As a further exploratory analysis, a maximum rank statistical analysis (16) was used to determine if a lower bound of androstenedione existed above and below which survival differed, regardless of the median. This analysis was done to generate a hypothesis that low adrenal androgen levels correspond to a tumor phenotype refractory to hormonal therapy, such as ketoconazole. With this analysis, it was determined that <0.49 ng/mL, the median survival was 12.9 (7.77-19.6) months, and above this level, it was 19.3 (14.1-28.3; P = 0.012; Fig. 2). Twenty-eight (27%) of 103 patients had baseline values <0.49 ng/mL and 75 (73%) had baseline values above this number.

Discussion

This study suggests that a modest association exists between pretreatment levels of androstenedione, an adrenal androgen, and response to ketoconazole, as well as overall survival in patients with AIPC who are treated with ketoconazole. These data, from a multicenter randomized trial, contrast with prior data from a smaller trial of 28 patients, which showed no correlation between baseline levels of any adrenal androgens and response to ketoconazole (13).

In the present analysis, baseline levels of these adrenal androgens in patients treated with ketoconazole initiated at the time of AAWD (arm 2) were studied and correlated to clinical outcomes. The primary focus was the ketoconazole arm of the study because this drug exerts its effect through suppression of adrenal androgens and because, in a prior analysis of the same data set, no change in adrenal androgen levels was observed over time in the AAWD alone arm (6). All demographic and baseline clinical data from the patients randomized to arm 2 were similar to those patients randomized to arm 1 and the group as a whole.

Of the adrenal androgens measured at baseline, only androstenedione levels were associated with a statistically significant degree with a 50% decline in PSA following therapy with ketoconazole. Levels of the other androgens measured (dehydroepiandrostenedione, DHEAS, and testosterone) correlate with androstenedione levels (Table 4) but do not have independent associations with PSA response. Those patients who experienced a 50% decline in PSA in response to ketoconazole had significantly higher baseline levels of androstenedione when compared with those who did not respond (0.88 versus 0.53 ng/mL; P = 0.034). These data suggest that a higher androstenedione level may identify patients more likely to benefit from ketoconazole therapy.

Despite the fact that median androstenedione levels were one third and one five hundredth that of dehydroepiandrostenedione and DHEAS, they carried the most predictive significance in this analysis. It is not completely clear why androstenedione levels, and not dehydroepiandrostenedione or DHEAS levels, were associated with improved responses to ketoconazole. However, androstenedione is the immediate precursor of

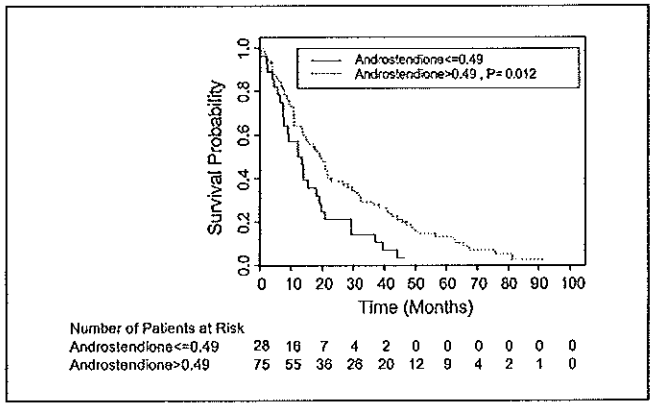

Fig. 2. Maximum rank analysis of the cutoff value of baseline androstenedione leading to differential survival in patients treated with AIPC treated with ketoconazole on CALGB 9583. There were 28 patients with values <0.49 and 75 patients above this value.

testosterone in the adrenal steroid synthesis pathway and this value may therefore be of physiologic importance. Further, androstenedione may itself bind to the AR, or be converted, through the intermediate formation of 5-androstane-3,17-diol (3-diol; androstenedione), to dihydrotestosterone, the androgen that most avidly binds to and activates the AR. The recent publications by Titus et al. (17) and Stanbrough et al. (4) show that tumors progressing despite androgen deprivation may contain measurable concentrations of adrenal androgens as well as express the enzymes responsible for the conversion of adrenal androgens to testosterone, respectively. These important observations suggest that perhaps conversion to testosterone within tumor cells is required and that direct agonism of AR by androstenedione may be only one of the mechanisms through which adrenal androgens promote tumor growth. Alternatively, it is possible that associations of outcome with other adrenal androgen levels were not observed because of inadequate sample size.

In addition to the association between androstenedione levels and response to therapy with ketoconazole, the relationship between baseline androstenedione levels and overall survival was studied. In multivariate analysis, patients with a baseline level of androstenedione above the median had improved survival when compared with those with lower androstenedione levels (HR, 0.64; 95% CI, 0.41-0.98; P = 0.04). This correlation suggests that the ability of ketoconazole to reduce levels of adrenal androgens may positively affect the outcome of patients with disease that is activated by circulating androstenedione (or other androgens in the microenvironment).

Despite this difference in HR for survival based on the baseline androstenedione level, when the survival of patients stratified by the median androstenedione level, no difference was seen. These results indicate that confounding variables may carry greater prognostic weight than does baseline hormonal status. In response to this, an analysis of potentially confounding variables was done and is shown in Table 4. In this analysis, no imbalance is observed in the age, Gleason score, performance status, or alkaline phosphatase among the three tertiles. A significant difference in LDH was seen among the tertiles, again consistent with more rapidly progressing disease in patients with lower androgen levels.

Because of the nonlinear association between baseline androstenedione level and survival, the maximum rank statistic (16) was used in a purely exploratory fashion to determine if a cutoff value existed around which outcomes were significantly different, potentially accounting for confounding variables. With this analysis, it was determined that a cutoff value (0.49 ng/mL), which falls well below the median (0.64 ng/mL) but only slightly above the lowest tertile cutoff (0.50 ng/mL), resulted in the greatest difference in survival (median, 12.9 versus 19.3 months; P = 0.012). Whether this value can be used for guiding therapeutic decisions with regard to secondary hormonal therapy requires further study, but it supports the hypothesis that progression of disease in the face of low androgen levels is a poor prognostic feature.

The relationship between androgen levels and survival is seen most significantly in the patients treated with immediate ketoconazole and is also evident in a multivariate analysis of all 197 patients who received ketoconazole on the study (accounting for crossover). Interestingly, in arm 1, patients with a higher androstenedione level were less likely to experience an AAWD response (HR, 0.40; 95% CI, 0.01-0.18; P = 0.04).

Patients in the lower third of baseline androstenedione levels had a significantly shorter survival (13.9 months) when compared with those in the middle (21.2 months; HR, 0.53; P = 0.018) or highest (15.7 months; HR, 0.59; P = 0.04) tertiles. The decreased survival in patients with the lowest androstenedione levels is consistent with a tumor phenotype that is truly hormone "refractory," capable of growth in a near complete absence of androgen. It is also noteworthy that these patients had consistently lower levels of other androgens and a higher median level of LDH (possibly reflecting more rapidly dividing tumors). Conversely, those patients with higher levels of androstenedione have more substrate for the pharmacologic effects of ketoconazole and by extension may have an improved survival because of the ability of ketoconazole to reduce androstenedione levels. An alternative hypothesis is that patients with a more aggressive tumor phenotype may have, as a consequence of their morbidity, reduced adrenal function and therefore lower androgen levels. In either case, these data suggest that a distinction may exist between prostate cancer that is only androgen independent ("testosterone independent" may be even more descriptive) and hormone "refractory."

It is not clear why patients in the middle androstenedione tertile had best overall survival. Interestingly, there was no significant association between PSA response and overall survival when stratified by tertile, which suggests that the association between levels and survival may not be a reflection of an association between hormone levels and response to ketoconazole.

A review of all potential confounding variables failed to show a clear pattern that can explain this association. The recent evidence that metastatic tumors from patients with hormone-refractory prostate cancer may convert androgens suggests that perhaps a higher tumor burden in some patients may lead to a higher circulating androgen burden as well, which could lead to a worse outcome for patients with higher serum androgen levels, and thus confound this data set. Further study of the relationship between serum androgens and tumor androgen production is required to formally test this hypothesis.

As stated above, recently published literature suggests that AIPC is associated with elevated intratumoral levels of androgens (17) as well as the expression of genes that are associated with the conversion of adrenal androgens to testosterone (4). These interesting observations raise the possibility that adrenal androgen level may increase independent of adrenal function, an area that clearly requires further study. The fact that several studies (6, 13, 18) have shown that ketoconazole can decrease adrenal androgen levels raises the question of whether ketoconazole actually exerts its effect in the adrenal gland or within the tumor itself. Further, the possibility that hydrocortisone may reduce adrenal function through pituitary suppression, or perhaps directly on the tumor cells, bears mention as a potential confounding variable that will require further study.

The effect of ketoconazole therapy on adrenal androgen levels at the 1-month, 3-month, and time of disease progression time points was recorded and has been published previously(6). At baseline, the median levels of DHEAS and androstenedione

were within the reference range, whereas baseline levels of dehydroepiandrostenedione were elevated slightly. No significant change in the levels of any adrenal androgens was observed over time in the AAWD alone arm of the study (arm 1). By contrast, in those patients treated with ketoconazole simultaneous with AAWD, there was a decline of dehydroepiandrostenedione, DHEAS, and androstenedione by 54%, 90%, and 58%, respectively, after 1 month of therapy. At the time of clinical progression, a statistically significant increase in the level of these androgens was observed; however, they did not return to baseline. One potential interpretation of these data is that defects in ketoconazole bioavailability, rather than the emergence of a tumor-resistant phenotype, may result in a loss of its ability to suppress adrenal androgen production after prolonged therapy.

Conclusion

These data suggest that baseline levels of the adrenal androgen androstenedione are modestly predictive of clinical outcomes in patients with AIPC.

Future work will seek to further clarify those patients who are most likely to benefit from ketoconazole and to target the mechanisms of eventual resistance to ketoconazole therapy. Whether pretreatment androstenedione levels can be used to develop risk-adapted therapy, in which patients with high androstenedione levels receive ketoconazole, whereas those patients with low androstenedione levels receive other therapy, requires prospective validation. Although these data do not show a strong enough link between higher baseline values and response to ketoconazole, they suggest that patients with disease progression and low levels of adrenal androgens have a worse prognosis and may not benefit from secondary hormonal therapy, such as ketoconazole.

Appendix 1. Institutions that participated in this study

(*1*) CALGB Statistical Center, Duke University Medical Center, Durham, NC–Stephen George, Ph.D. (grant CA33601, CA47577)
(*2*) CALGB Statistical Office, Durham, NC–Stephen George, Ph.D. (grant CA33601)
(*3*) Dartmouth Med School-Norris Cotton Cancer Center, Lebanon, NH–Marc S. Ernstoff, M.D. (grant CA04326)
(*4*) Georgetown University Medical Center, Washington, DC–Edward Gelmann, M.D. (grant CA77597)
(*5*) Illinois Oncology Research Association, Peoria, IL–John W. Kugler, M.D. (grant CA35113)
(*6*) Mount Sinai School of Medicine, New York, NY–Lewis R. Silverman, M.D. (grant CA04457)
(*7*) Rhode Island Hospital, Providence, RI–Louis A. Leone, M.D. (grant CA08025)
(*8*) Roswell Park Cancer Institute, Buffalo, NY–Ellis Levine, M.D. (grant CA02599)
(*9*) SUNY Upstate Medical University, Syracuse, NY–Stephen L. Graziano, M.D. (grant CA21060)
(*10*) University of California at San Diego, San Diego, CA–Stephen L Seagren, M.D. (grant CA11789)
(*11*) University of California at San Francisco, San Francisco, CA–Alan P. Venook, M.D. (grant CA60138)
(*12*) University of Chicago Medical Center, Chicago, IL–Gini Fleming, M.D. (grant CA41287)
(*13*) University of Illinois Minority-Based Community Clinical Oncology Program, Chicago, IL–Thomas Lad, M.D. (grant CA74811)
(*14*) University of Iowa, Iowa City, IA–Gerald Clamon, M.D. (grant CA47642)
(*15*) University of Maryland Cancer Center, Baltimore, MD–Martin Edelman, M.D. (grant CA31983)
(*16*) University of Massachusetts Medical Center, Worcester, MA–Mary Ellen Taplin, M.D. (grant CA37135)
(*17*) University of Minnesota, Minneapolis, MN–Bruce A Peterson, M.D. (grant CA16450)
(*18*) University of Missouri/Ellis Fischel Cancer Center, Columbia, MO–Michael C Perry, M.D. (grant CA12046)
(*19*) University of Nebraska Medical Center, Omaha, NE–Anne Kessinger, M.D. (grant CA77298)
(*20*) University of Tennessee Memphis, Memphis, TN–Harvey B. Niell, M.D. (grant CA47555)
(*21*) Vermont Cancer Center, Burlington, VT–Hyman B. Muss, M.D. (grant CA77406)
(*22*) Wake Forest University School of Medicine, Winston-Salem, NC–David D Hurd, M.D. (grant CA03927)
(*23*) Walter Reed Army Medical Center, Washington, DC–Joseph J. Drabeck, M.D. (grant CA26806)
(*24*) Washington University School of Medicine, St. Louis, MO–Nancy Bartlett, M.D. (grant CA77440)
(*25*) Weill Medical College of Cornell University, New York, NY–Michael Schuster, M.D. (grant CA07968)
(*26*) Dana-Farber Cancer Institute, Harvard University, Boston, MA–Eric Wiher (grant CA32291)

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