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ORIGINAL REPORT

Serum Androgens As Prognostic Biomarkers in Castration-Resistant Prostate Cancer: Results From an Analysis of a Randomized Phase III Trial

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

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ABSTRACT

Purpose
In the phase III study COU-AA-301, abiraterone acetate (AA) plus prednisone (P) prolonged overall survival (OS) in patients with metastatic castration-resistant prostate cancer (mCRPC) after docetaxel administration. In this article, we investigate the relationship between baseline serum androgen (SA) levels and OS.

Patients and Methods
COU-AA-301 is a randomized, double-blind study of AA (1,000 mg every day) plus P (5 mg by mouth twice daily; n = 797) versus P alone (n = 398). Randomization was stratified by Eastern Cooperative Oncology Group performance status (0 to 1 *v* 2), pain (Brief Pain Inventory-Short Form over past 24 hours: 4 to 10, present; *v* 0 to 3, absent), prior chemotherapy (1 *v* 2), and progression (prostate-specific antigen *v* radiographic). Association of baseline SA (testosterone, androstenedione, dehydroepiandrosterone sulfate), was measured by ultrasensitive liquid-liquid extraction or protein precipitation and two-dimensional liquid chromatography coupled to mass spectrometry, with OS determined by bivariate and multivariable Cox models. OS was examined with SA as greater than median and less than or equal to the median.

Results
Median survival increased with each quartile increase in testosterone level regardless of treatment arm. SA levels at baseline strongly associated with survival ($P < .0001$) in bivariate and multivariable analyses. Longer survival was observed for patients with SA above median compared with below median in both the AA and P arms (eg, testosterone, AA; hazard ratio, 0.64; 95% CI, 0.53 to 0.77; $P < .0001$). Treatment with AA led to longer survival versus P alone in the above- or below-median group for all androgens.

Conclusion
SA, measured with a novel ultrasensitive assay in COU-AA-301, is prognostic for OS and may be useful for risk stratification in mCRPC clinical trials.

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INTRODUCTION

Metastatic castration-resistant prostate cancer (mCRPC) remains sensitive to further manipulations of the androgen receptor (AR) signaling cascade as shown by abiraterone acetate (AA), an oral androgen biosynthesis inhibitor of the cytochrome P450 c17 (*CYP17*) enzyme complex.[1-4] The phase III study COU-AA-301 (Abiraterone Acetate in Metastatic Castration-Resistant Prostate Cancer Previously Treated With Docetaxel-Based Chemotherapy), of patients with mCRPC experiencing disease progression after chemotherapy, demonstrated increased rates of overall survival (OS) with AA plus prednisone (P) versus P alone, confirming that suppression of androgen production beyond that with luteinizing hormone–releasing hormone (LHRH) analogs led to a clinical benefit.[5]

Nongonadal sources of testosterone include the adrenal glands and prostate cancer cells through intracrine production, both of which contribute to disease progression despite castrate levels of testosterone.[6-8] Androgen-deprivation therapy with orchiectomy or LHRH analogs reduces testicular androgen production without affecting adrenal or intracrine androgen synthesis.[9] In castration-resistant disease, extragonadal synthesis produces tumor androgen levels exceeding those in the prostates of eugonadal men that are sufficient to activate AR signaling.[10,11]

Androgens, such as androstenedione and dehydroepiandrosterone sulfate (DHEAS), are AR agonists that may affect disease progression.[12] These androgens and testosterone have been the target of therapeutic trials with corticosteroids[13] and ketoconazole.[14] Higher androstenedione levels were associated with prostate-specific antigen (PSA) decline in ketoconazole plus hydrocortisone–treated patients.[15] In vitro data suggest that adrenal and intratumoral androgens may confer resistance to androgen synthesis inhibitor therapy.[6]

Higher baseline serum testosterone, and precursors DHEAS and androstenedione, may be prognostic by identifying mCRPC patients with tumors that may be more dependent on androgens for growth regardless of the source. To test this hypothesis, a retrospective analysis of COU-AA-301was conducted to define the distribution of baseline serum androgen concentrations and to evaluate their association with OS.

PATIENTS AND METHODS

Patients

COU-AA-301 was a multinational, randomized, double-blind study of AA (1,000 mg once daily) plus P (5 mg by mouth twice a day) versus P alone (treatment arms are hereafter referred to as AA or P, respectively) in patients with mCRPC after receiving docetaxel (Fig 1). Patients had evidence of progressive disease after orchiectomy or ongoing medical castration with an LHRH analog. Patients were stratified by Eastern Cooperative Oncology Group performance status (0 to 1 *v* 2), worst pain over the past 24 hours on the Brief Pain Inventory-Short Form (0 to 3, absent; *v* 4 to 10, present), one versus two prior chemotherapy regimens, and type of progression (PSA progression only *v* radiographic progression with or without PSA progression).

The review boards at all participating institutions approved the study, which was conducted according to the Declaration of Helsinki. All patients provided written, informed consent to participate in the study.

Androgen Assays

Quantitative bioanalytical methods were developed and validated to determine testosterone, androstenedione, and DHEAS levels in human serum using liquid-liquid extraction or protein precipitation and two-dimensional liquid chromatography coupled to tandem mass spectrometry [(LC)-LC-MS/MS; Endocrine Sciences, Laboratory Corporation of America, Calabasas Hills, CA]. Stable isotope internal standards were used to account for any losses during processing. Testosterone and androstenedione were extracted from serum samples with a hexane:ethyl acetate mixture to separate these androgens from binding proteins and interferents. After evaporation and reconstitution, samples were analyzed by LC using an ARIA Transcend TX4 system (Thermo Fisher, Franklin, MA). An MDS-Sciex API5000 triple quadruple mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA) operating in positive ion atmospheric pressure chemical ionization mode was used for detection. Quantification of analyte and internal standard was performed in selected reaction monitoring mode. The back-calculated amount of androgen in each sample was determined from duplicate calibration curves generated by spiking known amounts of purified androgen into diluted charcoal-stripped female human serum from 0.05 to 100 ng/dL for testosterone and from 0.1 to

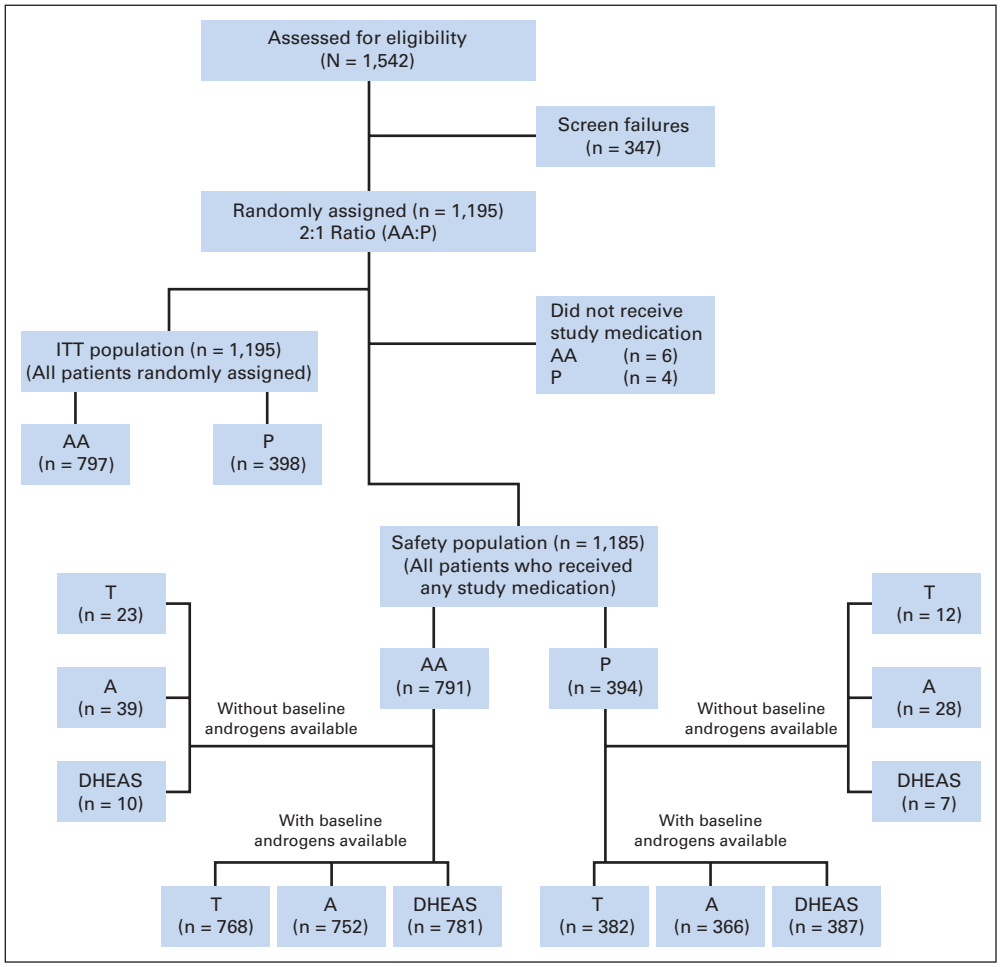

Fig 1. CONSORT diagram. A, androstenedione; AA, abiraterone acetate; DHEAS, dehydroepiandrosterone sulfate; ITT, intent-to-treat; P, prednisone; T, testosterone.

100 ng/dL for androstenedione. DHEAS was measured by MS detection after protein precipitation. Stable labeled heavy isotope DHEAS was added as an internal standard to serum aliquots. Analysis was performed using LC separation with tandem mass spectrometric detection (LC-MS/MS). An MDS-Sciex API5000 triple quadrupole mass spectrometer, operating in negative ion electrospray ionization mode, was used for detection. Analyte and internal standard quantification was performed in selected reaction monitoring mode.

The back-calculated amount of DHEAS in each sample was determined from duplicate sets of calibrators generated by spiking known amounts of purified DHEAS into a clean blank matrix prepared from diluted charcoal stripped serum from 0.1 to 10 μg/dL. Performance characteristics of the assays include within- and between-run accuracy of 85% to 115%, within- and between-run precision less than 15%, recoveries between 85% and 115%, and linearity, as defined by correlation coefficients (r^2), greater than 0.995 throughout the analytic measurable range. Assay validation is provided in the Appendix (online-only; Appendix Table A1; Appendix Fig A1). There was no assay interference from AA and 46 steroid hormones in the quantitation of each androgen.

Statistical Design and Data Analysis

Pearson correlation was used to compute the correlation between each pair of androgens. To determine the association between androgens and the efficacy end points, bivariate and multivariable Cox models were used for OS. A logistic model was used for the PSA response (defined as a $\geq$ 50% decrease in PSA concentration from the pretreatment PSA value, confirmed at least 4 weeks later by an additional PSA evaluation). The models included treatment and each androgen independently; test of the interaction effect was conducted within the same model. All analyses were adjusted for the baseline stratification factors. Baseline laboratory variables were dichotomized as greater than median or less than or equal to median for analysis of lactate dehydrogenase (LDH), hemoglobin, alkaline phosphatase (ALP), PSA, and serum androgens. The multivariable model included treatment, androgens, and other dichotomized laboratory parameters (ie, LDH, hemoglobin, ALP, and PSA). The Cox proportional hazards model was used to determine the association between androgens and OS. Median survival was estimated using the Kaplan-Meier method. The relationship between baseline serum androgen levels and OS was also evaluated by smoothed Kaplan-Meier estimates of the median conditional survival time; androgens were log-transformed before that analysis. In the PSA response analysis, logistic regression was used to obtain odds ratios with the factors described herein. Because of the exploratory nature of these analyses, significance was declared if $P \leq .05$ without adjusting for multiplicity testing.

Table 1. Summary of Baseline Androgen Levels in Patients Treated With AA Plus P or Placebo Plus P

Androgen	AA (n = 791)	P (n = 394)	Total (N = 1,185)
Testosterone, ng/dL			
No. of patients	768	382	1,150
Mean	7.6	6.9	7.4
SD	17.85	11.67	16.06
Median	5.1	4.8	5.0
Range, min to max*	0.1 to 309.0	0.1 to 185.0	0.1 to 309.0
Androstenedione, ng/dL			
No. of patients	752	366	1,118
Mean	30.9	30.3	30.7
SD	29.47	27.06	28.7
Median	24.2	22.9	23.7
Range	0.0 to 317.0	0.51 to 173.0	0.40 to 317.0
DHEAS, μg/dL			
No. of patients	781	387	1,168
Mean	25.9	28.1	26.6
SD	30.81	32.28	31.3
Median	15.9	16.2	16.0
Range	0.1 to 230.0	0.2 to 180.0	0.1 to 230.0

Abbreviations: AA, abiraterone acetate; DHEAS, dehydroepiandrosterone sulfate; min, minimum; max, maximum; P, prednisone; SD, standard deviation.

*There were two patients enrolled onto the study with baseline androgen levels $\geq$ 50 ng/dL despite the eligibility requirement of $<$ 50 ng/dL.

RESULTS

From May 2008 to July 2009, 1,195 patients were enrolled onto the COU-AA-301 trial and were randomly assigned at a 2:1 ratio to the AA (n = 797) or P (n = 398) treatment arms (Fig 1). Primary results were reported previously.[5]

Ninety percent of patients entering the study had bone metastases and 30% had visceral metastatic disease. The median survival rate for patients in the updated analysis was 15.8 months for the AA arm versus 11.2 months for the P arm.[16] The proportion of patients with a $\geq$ 50% PSA decline was higher in the AA arm compared with the P arm (29.1% *v* 5.5%; $P < .0001$).

Of the 1,185 patients who received treatment (AA, n = 791; P, n = 394), baseline androgen levels as measured by the ultrasensitive

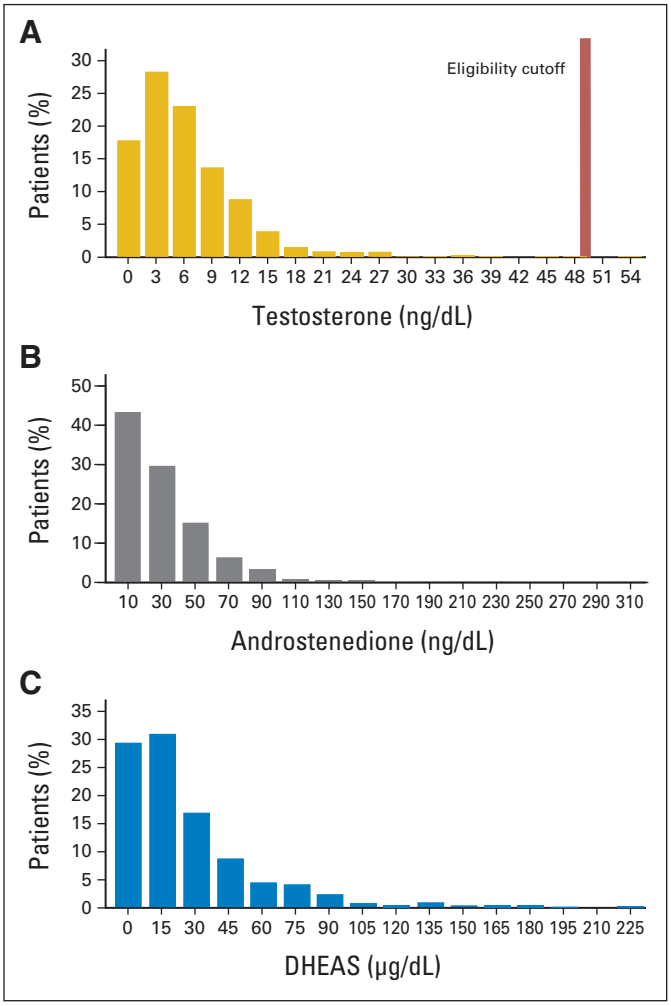

Fig 2. Distribution of serum androgen levels at baseline in patients with metastatic castration-resistant prostate cancer enrolled onto the COU-AA-301 phase III trial. (A) Testosterone, (B) androstenedione, and (C) dehydroepiandrosterone sulfate (DHEAS).

assays were available for 768 (97%), 752 (95%), and 781 (99%) patients, respectively, in the AA arm, and 383 (97%), 366 (93%), and 387 (98%) patients, respectively, in the P arm. The median levels of baseline testosterone, androstenedione, and DHEAS were 5.0 ng/dL, 23.7 ng/dL, and 16 μg/dL, respectively, and were similar in the AA and P cohorts. Baseline androgen levels and their ranges are listed in Table 1. Ninety-three percent of enrolled patients had baseline testosterone levels below 10 ng/dL, which was well below the 50 ng/dL eligibility cutoff. The distribution of baseline androgens is shown in Figure 2. All three androgens were positively correlated with one another (Appendix Table A2). The correlation coefficient for testosterone to androstenedione was 0.30, for testosterone to DHEAS 0.18, and for androstenedione to DHEAS 0.45 (all $P < .0001$).

Median survival increased from first quartile (lowest) to fourth quartile (highest) of testosterone, regardless of treatment arm (Fig 3). The median survival by testosterone quartile Q1 (≤ 2.3 ng/dL), Q2 (> 2.3 to ≤ 5.0 ng/dL), Q3 (> 5.0 to ≤ 8.6 ng/dL), and Q4 (> 8.6 ng/dL) was 10.4 (95% CI, 8.8 to 11.6), 13.3 (95% CI, 11.2 to 14.9), 16.1 (95% CI, 14.8 to 18.0), and 18.9 (95% CI, 16.6 to not estimable) months, respectively ($P < .0001$). Similar results were observed for androstenedione and DHEAS (Appendix Table A3).

Survival curves based on testosterone level stratified at the median and treatment arms (AA *v* P) are shown in Figure 4A. The longest median survival rate was observed in patients with baseline testosterone above the median when treated with AA—approximately 18.0 months, an outcome that was consistent across the three androgens (Fig 4 and Table 2). Comparing within AA or within P, a reduction in the risk of death was observed in the group of patients with baseline androgen above the median compared with baseline androgen below the median. Comparing patients treated with AA versus P, in groups with baseline androgen above the median or those with baseline androgen below the median, the risk reduction in death consistently favored the AA arm. Improvement with AA versus P was observed for all androgens in the below-median group and for DHEAS in the above-median group, whereas a favorable risk reduction was observed for both testosterone and androstenedione in the above-median group. Median survival was longer with increasing levels of testosterone and DHEAS, although the trend was less clear with androstenedione (Appendix Fig A2). A similar association was observed with consideration of each of the androgens as continuous variables.

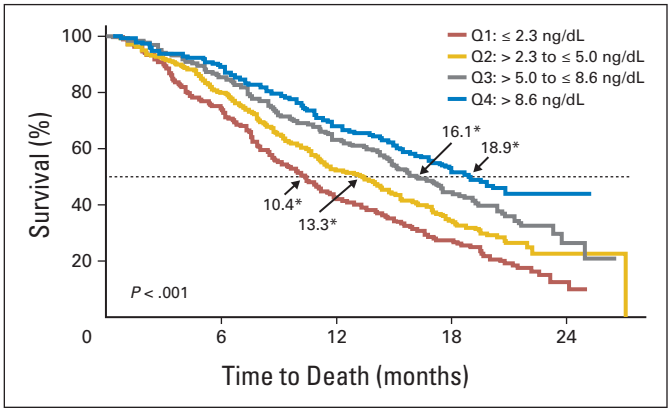

Fig 3. Overall survival as a function of baseline testosterone stratified by quartiles (Q). (*) Median overall survival in months. All analyses were adjusted by baseline stratification factors.

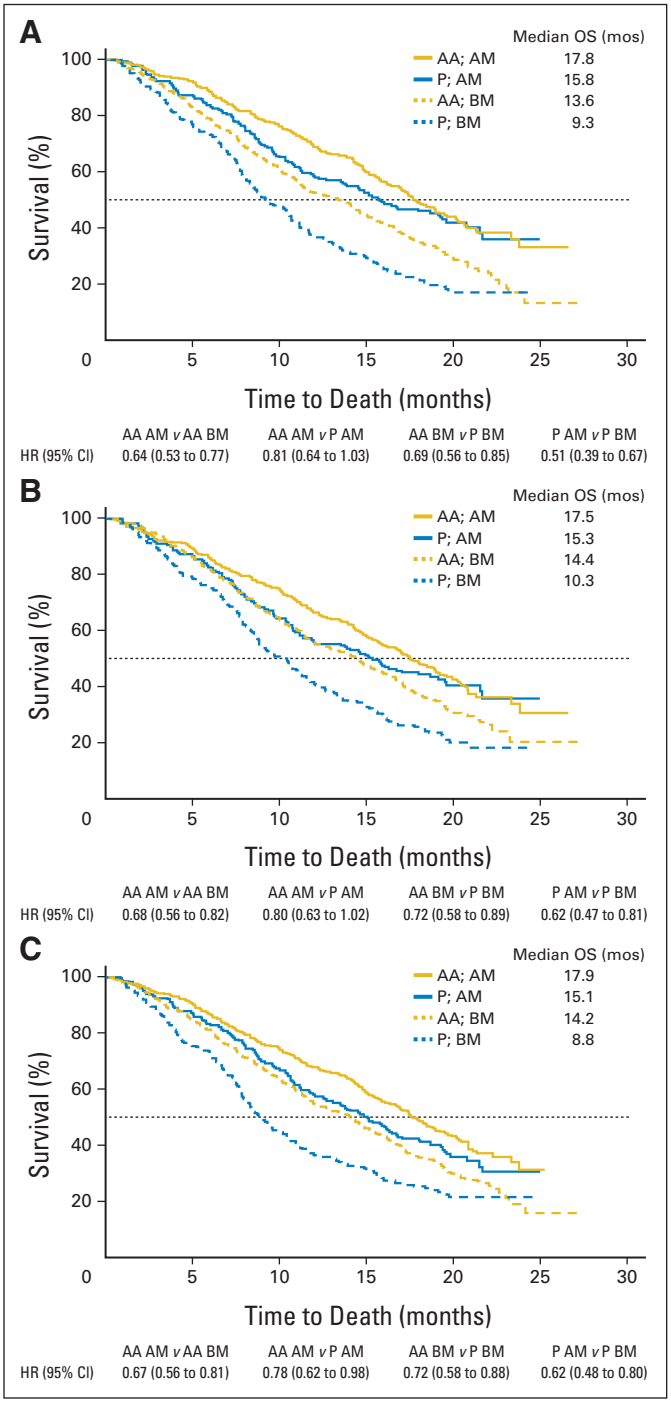

Fig 4. Overall survival (OS) as a function of baseline androgen status stratified above median (AM) or below median (BM) in patients treated with abiraterone acetate (AA) plus prednisone (P) or placebo plus P. (A) Testosterone, (B) androstenedione, and (C) dehydroepiandrosterone sulfate. All analyses were adjusted by baseline stratification factors. HR, hazard ratio; mos, months.

Patients with above-median baseline serum androgens were associated with improved OS and better PSA response rate irrespective of bivariate or multivariable analyses that account for the effect of known prognostic laboratory parameters of LDH, hemoglobin, ALP, and PSA (Table 3). The median levels of LDH, hemoglobin, ALP, and PSA were 227 IU/mL, 11.8 g/dL, 134 IU/L, and 131.4 ng/mL,

Table 2. Overall Survival in Patients Treated With AA or Placebo Plus P Based on Baseline Androgen Levels AM or BM

Androgen	Median Overall Survival (months)				AA AM *v* AA BM			AA AM *v* P AM			AA BM *v* P BM			P AM *v* P BM		
	AA AM	AA BM	P AM	P BM	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Testosterone	17.8	13.6	15.8	9.3	0.64	0.53 to 0.77	< .001	0.81	0.64 to 1.03	.0834	0.69	0.56 to 0.85	< .001	0.51	0.39 to 0.67	< .001
Androstenedione	17.5	14.4	15.3	10.3	0.68	0.56 to 0.82	< .001	0.80	0.63 to 1.02	.0736	0.72	0.58 to 0.89	.0019	0.62	0.47 to 0.81	< .001
DHEAS	17.9	14.2	15.1	8.8	0.67	0.56 to 0.81	< .001	0.78	0.62 to 0.98	.0343	0.72	0.58 to 0.88	.0015	0.62	0.48 to 0.80	< .001

NOTE. Not all patients were included in this analysis, and the survival data may be slightly different from that of the intent-to-treat analysis. All analyses were adjusted by baseline stratification factors.

Abbreviations: AA, abiraterone acetate; AM, above the median; BM, below the median; DHEAS, dehydroepiandrosterone sulfate; HR, hazard ratio; P, prednisone.

respectively. In the bivariate and multivariable analyses, treatment effect was consistent among all of the different androgens tested.

DISCUSSION

These data from a randomized phase III study demonstrate that baseline serum testosterone, measured with ultrasensitive techniques, is prognostic of OS in patients with mCRPC independent of treatment arm. As a serum-based biomarker that is prognostic for survival, testosterone levels may be a factor that affects study outcomes and suggests that the role of ultrasensitive testosterone levels should be further investigated for patient management and in clinical trials. These results are consistent with the hypothesis that disease progression in a very low testosterone milieu represents a distinct and more aggressive biologic and clinical state associated with decreased survival versus survival of patients who harbor a form of the disease that remains more dependent on stimulation by circulating androgen.

Table 3. Higher Baseline Androgens Are Associated With Improved Overall Survival and PSA Response Rate by Bivariate and Multivariable Analysis

Androgen	Overall Survival		PSA Response Rate	
	HR	*P*	OR	*P*
Bivariate				
Testosterone	0.595	< .001	0.376	< .001
Treatment/testosterone interaction	1.213	.2239	1.202	.7147
Androstenedione	0.660	< .001	0.391	< .001
Treatment/androstenedione interaction	1.141	.4144	0.928	.8876
DHEAS	0.653	< .001	0.391	< .001
Treatment/DHEAS interaction	1.107	.5170	1.624	.3204
Multivariable*†‡				
Testosterone	0.667	< .001	0.405	< .001
Androstenedione	0.679	< .001	0.408	< .001
DHEAS	0.691	< .001	0.411	< .001

Abbreviations: ALP, alkaline phosphatase; DHEAS, dehydroepiandrosterone sulfate; HR, hazard ratio; LDH, lactate dehydrogenase; OR, odds ratio; PSA, prostate-specific antigen.

*Including one androgen at a time (dichotomized) and other dichotomized laboratory parameters (LDH, hemoglobin, ALP) in the model.

†Besides study treatment and androgen, the relative contribution of the variables was in the following order: LDH, hemoglobin, ALP, and PSA. For the overall survival end point, the relative significant contribution of the variables was in the following order: LDH, androgen, study treatment, hemoglobin, ALP, and PSA. For PSA response, the relative significant contribution of the variables was in the following order: study treatment, androgen, and LDH.

‡Interaction between treatment and androgen in the multivariable analyses was not significant ($P > .05$).

In addition to testosterone, a similar relationship was observed with androstenedione and DHEAS, and a positive correlation ($P < .0001$) among all androgens was noted (Appendix Table A2). These data suggest that there is no single dominant androgen responsible for this association, although androgen receptor binding avidity may vary. As testosterone precursors, continued assessment of these levels may be useful in determining the effect of total serum androgen on patient outcomes. To date, no models exist that account for total androgen load as opposed to the measurement of the level of an individual hormone (eg, testosterone).

Treatment with AA proved beneficial over P at all levels of androgen measured. The most pronounced treatment effect of AA over P was observed in patients with lower androgens (Table 2). This may reflect heightened sensitivity of tumors to very low androgen levels or that a low serum androgen milieu may be distinct from a low intratumoral androgen milieu. Patients with baseline androgen levels above the median who were treated with AA had the longest median survival of all groups (17.5 to 17.9 months) and survived longer than patients with androgen levels below the median who received AA (13.6 to 14.4 months).

The outcome of longer survival among patients with baseline androgen levels above the median was observed with each androgen evaluated. Although AA increased OS over P alone in the above-median androgen group for DHEAS, this was not observed for testosterone and androstenedione. The trends favored AA and suggest that changes in androgen levels may be more critical than baseline levels in patients with higher levels. The relationship of decline in androgen levels with PSA decline is the subject of ongoing analysis.

A positive association between baseline testosterone level and OS was observed after adjusting for treatment, other androgens, and other laboratory parameters (ie, LDH, hemoglobin, ALP, PSA). This demonstrates, somewhat surprisingly, that incomplete suppression of testosterone by medical or surgical castration may be a prognostic factor that is independent of other prognostic factors that reflect advanced disease or end organ involvement and is a prognostic factor that can be readily measured with commercially available assays. Further, it suggests that, as an unrecognized contributor to patient outcome, androgen level variations may have confounded previous large clinical trial results.

Maintenance of a castrate testosterone level is required in virtually all studies in mCRPC, yet, at present, little is known about testosterone level variability in patients who have undergone medical castration with an LHRH analog. By consensus, a testosterone level of less than 50 ng/dL was defined as castrate,[17] based on the insensitivity

of the assays available when the Prostate Cancer Clinical Trials Working Group 2 guidelines were developed. Novel assays enable more precise androgen quantification in the previously defined "castrate" range to a sensitivity of 0.05 ng/dL and the opportunity to explore their relationship to outcome.[18-21]

The ultrasensitive LC/LC-MS/MS assays used in this study allow for the assessment of androgen levels below traditionally defined castrate levels. In 12 patients with a rising PSA on LHRH agonist therapy using the identical ultrasensitive assay,[22] pretreatment serum testosterone levels ranged from 1.69 to 6.39 ng/dL (mean, 3.86 ng/dL) compared with a range of less than 1 to 6 ng/dL (mean, 2.7 ng/dL) obtained by a commercially available assay (Quest Diagnostics). The commercially available assay did not account for variability in values less than 1 ng/dL (lower limit of quantification, 1.0 ng/dL), whereas those obtained by the ultrasensitive assay were more precise, showing accurate and reproducible measurement of serum testosterone levels despite a six-fold reduction in concentration. Categorization of results in the form of quartiles and at the median, as was done in our study, may offer convenient and clinically accessible values to stratify patients in future studies by androgen levels or to allocate risk groups across discrete cut points.

The availability of an ultrasensitive assay coupled with the demonstrated prognostic significance of baseline testosterone levels mandates the routine use of the ultrasensitive assays in clinical trials. Instead of assigning an arbitrary cutoff of total testosterone less than 50 ng/dL, as used previously with distinct assay techniques,[17,23] stratification of patients by androgen levels may lead to more balanced patient allocation, particularly for agents that act by inhibiting androgen signaling.

Given the use of an active control, validation of these results in patients treated without prednisone is warranted. Similarly, validation of the prognostic-predictive nature of serum testosterone in mCRPC patients who are chemotherapy-naive or have not yet developed metastases would be of value. Stratification may require more widespread adoption of the ultrasensitive (LC)-LC-MS/MS assay techniques, as in our study. Failure to do so could potentially lead to imbalanced treatment arms and confounding results for agents that target persistent androgen production or signaling in mCRPC.

The cause of variation in androgen levels is not understood, although polymorphisms in the *CYP17* gene and other androgen-regulating enzymes have been proposed and have been associated with prostate cancer outcomes.[24-26] Such heritable factors, and the differential potency of LHRH antagonists or agonists,[27] may affect the androgen levels when patients develop castration resistance via incomplete suppression and endogenous upregulation.

Though patients with below-median baseline androgens benefited from AA therapy (compared with P), they nevertheless had shorter survival rates compared with patients with above-median baseline androgens. This result is striking when viewed by quartiles (Fig 3). This population should be targeted for further evaluation and possible selection for trials that build on androgen synthesis inhibition and target additional mechanisms of resistance or other growth pathways, including PI3Kinase.[28] It is hypothesized that tumors with amplified AR may be hypersensitive to androgens,[7,29] which may explain why the survival in patients treated with AA was better than in those receiving P, even in patients with low androgen levels. Although numerous studies associate low testosterone levels with increased mortality from noncancer causes,[30-32] in our study, prostate cancer was the cause of death in virtually all patients.

These results from the COU-AA-301 study parallel those from a previous phase III study of ketoconazole, another androgen synthesis inhibitor.[15] Unlike the ketoconazole study, which showed an association between OS and androstenedione levels but not with DHEAS or testosterone, our current data suggest that any androgen may be prognostic. This may reflect a roughly four-fold higher COU-AA-301 sample size, relative to the prior ketoconazole study, or the greater sensitivity provided by the ultrasensitive assays employed in our study.

Several caveats should be considered when interpreting these data, including the fact that these analyses were exploratory, with no attempt to correct for multiplicity. Though adrenal and intratumoral de novo androgen synthesis contributes to disease progression,[10,11] the relationship between serum androgens and intratumoral androgens remains poorly understood. It remains unclear whether the relationship between higher baseline serum androgens and survival would be observed in earlier (chemotherapy-naive) mCRPC disease states or with other therapies that do not modulate androgen levels. Androgen levels may be affected by diet and circadian conditions, not well accounted for in our study. The fact that P may modulate androgen levels may be a confounder. These results will require further validation using an external data set.

In this randomized, phase III study (COU-AA-301) of AA versus P in mCRPC patients, baseline androgens as measured by a novel ultrasensitive assay may be prognostic of OS. Baseline androgen levels above the median were associated with increased survival relative to baseline androgens below the median in patients treated with either AA or P. These data may be useful in risk stratification of patients in future studies or in need of novel combination therapies. The relationship between serum and intratumoral androgen levels merits investigation as both may contribute to progression and morbidity in castration-resistant prostate cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
Employment or Leadership Position: Arturo Molina, Janssen Pharmaceuticals Research and Development (C); Jinhui Li, Janssen Pharmaceuticals Research and Development (C); Thian Kheoh, Janssen Pharmaceuticals Research and Development (C); Russell P. Grant, Laboratory Corporation of America (C); Johann S. de Bono, The Institute for Cancer Research (C) **Consultant or Advisory Role:** Johann S. de Bono, Astellas Pharma (C), Medivation (C), Johnson & Johnson (C); Howard I. Scher, Aragon Pharmaceuticals (U), Bristol-Myers Squibb (U), Celgene (U), Endo/Orion Pharmaceuticals (C), Exelixis (U), Foundation Medicine (U), Genentech (U), Janssen Pharmaceuticals (U), Johnson & Johnson Pharmaceutical and Development (U), Medivation (U), Millenium (U), Novartis (C), Ortho Biotech Oncology Research and Development (C), Takeda Millennium (U) **Stock Ownership:** Arturo Molina, Johnson & Johnson; Thian Kheoh, Johnson & Johnson; Christopher M. Haqq, Johnson & Johnson; Russell P. Grant, Laboratory Corporation of America **Honoraria:** Charles J. Ryan, Janssen Pharmaceuticals; Johann S. de Bono, Astellas Pharma, Medivation, Johnson & Johnson **Research Funding:** Howard I. Scher, Aragon Pharmaceuticals, Exelixis, Janssen Pharmaceuticals

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GLOSSARY TERMS

Abiraterone acetate: Selective inhibitor of androgen biosynthesis that potently blocks cytochrome P450c17 (CYP17).

Biomarker: A functional biochemical or molecular indicator of a biologic or disease process that has predictive, diagnostic, and/or prognostic utility.

Cytochrome P450 c17 (CYP17): A critical enzyme in testosterone synthesis with 17α-hydroxylase and C17, 20 lyase activities, which are necessary for the conversion of pregnenolone to 17α-hydroxypregnenolone and dehydroepiandrosterone and for the conversion of progesterone to 17α-hydroxyprogesterone, respectively.

DHEAS: Dehydroepiandrosterone sulfate, a metabolite of dehydroepiandrosterone (DHEA), an androgen produced by the adrenal gland.

(LC)-LC-MS/MS: Two-dimensional liquid chromatography coupled to tandem mass spectrometry.

mCRPC: Metastatic castration-resistant prostate cancer, progressive disease despite surgical castration or ongoing use of gonadotropin-releasing hormone agonists with confirmed castrate levels of testosterone.

Prognostic (prognostic marker): A marker that predicts the prognosis of a patient (eg, the likelihood of relapse, progression, and/or death) independent of future treatment effects. A factor can be both prognostic and predictive.

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Appendix

Table A1. Figures of Merit for Ultrasensitive LC-MS/MS Assays

Testosterone					
Calibrator concentration, ng/dL	0.2	2.5		50	100
Intraday precision, % CV, range	7.2-13.6	2.6-3.5		2.6-3.2	1.7-3.7
Interday precision, % CV, median	11.3	3.5		3.2	3.0
Intraday accuracy, %, range	90.7-99.9	98.7-103.1		95.9-99.5	96.0-99.8
Interday accuracy, %, median	96.1	100.4		97.4	98.2
Recovery, %			105.0-110.9		
Testosterone					
QC concentration, ng/dL	3	25		40	80
Intraday precision, % CV, range	3.1-13.3	2.9-3.5		1.3-3.3	2.4-3.4
Interday precision, % CV, median	10.6	3.2		2.5	3.2
Recovery, %			100.9-102.8		
Androstenedione					
Calibrator concentration, ng/dL	0.1	50		100	
Intraday precision, % CV, range	5.3-12.5	3.3-5.2		2.1-4.1	
Interday precision, % CV, median	10.2	5.1		3.8	
Intraday accuracy, %, range	94.0-98.0	97.1-104.3		100.5-105.8	
Interday accuracy, %, median	96.6	100.9		103.9	
Recovery, %		94.1-97.3			
Androstenedione					
QC concentration, ng/dL	0.5	8		60	90
Intraday precision, % CV, range	3.4-8.7	4.0-6.4		1.8-6.5	1.2-5.8
Interday precision, % CV, median	7.1	5.5		5.0	4.1
Recovery, %			100.9-102.8		
DHEAS					
Calibrator concentration, μg/dL	0.1	5		10	
Intraday precision, % CV, range	3.6-13.6	5.5-6.8		4.4-8.0	
Interday precision, % CV, median	8.2	6.4		6.1	
Intraday accuracy, %, range	101.8-105.8	101.1-106.3		96.7-102.0	
Interday accuracy, %, median	103.2	103.3		100.1	
Recovery, %		92.1-107.6			
DHEAS					
QC concentration, μg/dL	0.4	5		8	130*
Intraday precision, % CV, range	6.3-13.4	5.4-9.0		3.5-7.6	4.5-4.9
Interday precision, % CV, median	9.9	6.8		5.2	4.9
Recovery, %			86.4-100.9		

NOTE. Figures of merit represent measurement of calibrators prepared in charcoal stripped serum and pooled matrix for quality controls. Intraday studies represent the mean range of results from six replicates at each level for 3 days. Interday studies represent the mean results of all intrarun samples (n = 18). Recovery studies were performed in quadruplicate at three levels, two levels, and four levels for testosterone, androstenedione, and DHEAS, respectively.

Abbreviations: CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; LC-MS/MS, liquid-liquid extraction or protein precipitation and two-dimensional liquid chromatography coupled to mass spectrometry; QC, quality control.

*DHEAS QC at 130 μg/dL was diluted 20-fold into analytical measurement range prior to assay.

Table A2. Pearson Correlation Coefficient of Serum Androgens in Patients With Metastatic Castration-Resistant Prostate Cancer Treated in the COU-AA-301 Study

Androgen	Androstenedione	DHEAS
Testosterone		
r	0.29845	0.18049
P	< .001	< .001
No. of observations	1,104	1,146
Androstenedione		
r		0.44970
P	—	< .001
No. of observations		1,114

Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

Table A3. Median OS per Baseline Androgen Quartile

Androgen	Q1	Q2	Q3	Q4
Testosterone, ng/dL				
Quartile	≤ 2.3	> 2.3-≤ 5.0	> 5.0-≤ 8.6	> 8.6
Median OS, months	10.4	13.3	16.1	18.9
95% CI	8.8 to 11.6	11.2 to 14.9	14.8 to 18.0	16.9 to NE
Androstenedione, ng/dL				
Quartile	≤ 10.6	> 10.6-≤ 23.7	> 23.7-≤ 42.2	> 42.2
Median OS, months	11.1	14.3	17.0	17.0
95% CI	9.9 to 12.3	12.3 to 16.1	15.6 to 20.2	14.6 to 18.4
DHEAS, μg/dL				
Quartile	≤ 5.9	> 5.9-≤ 16	> 16-≤ 35	> 35
Median OS, months	10.6	13.7	14.9	18.9
95% CI	9.4 to 12.0	11.5 to 15.2	13.8 to 17.0	17.1 to 20.4

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; NE, not evaluable; OS, overall survival.

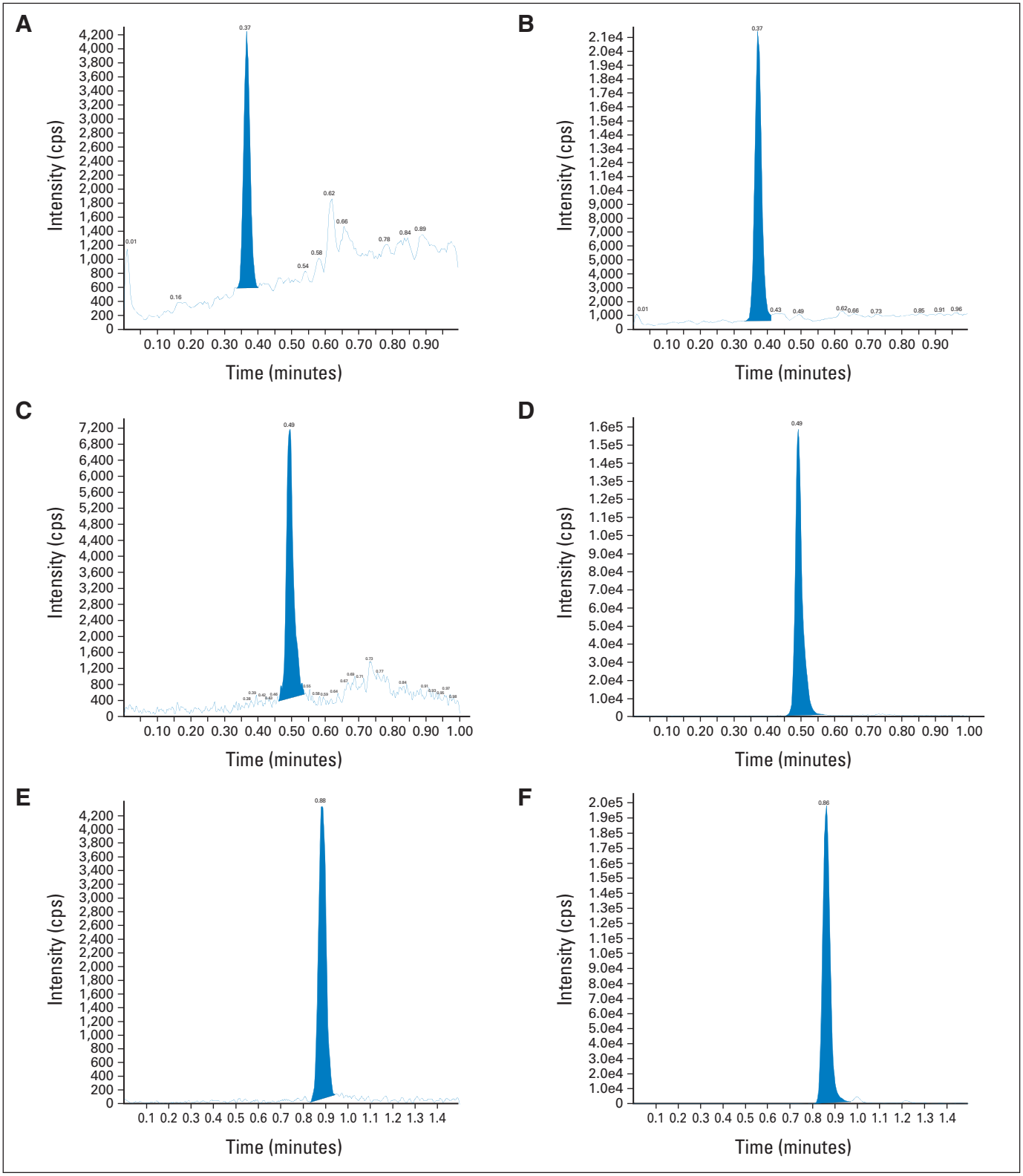

Fig A1. Representative liquid-liquid extraction or protein precipitation and two-dimensional liquid chromatography coupled to mass spectrometry chromatograms for testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS). (A), (C), and (E) represent calibrators at the lower limit of quantification for testosterone (0.2 ng/dL; signal to noise [S:N], 70), androstenedione (0.25 ng/dL; S:N, 102), and DHEAS (0.1 μg/dL; S:N, 494). (B), (D), and (F) represent specimens close to the Q1 androgen quartile for testosterone (2.5 ng/dL; S:N, 334), androstenedione (10.1 ng/dL; S:N, 3,355), and DHEAS (6.0 μg/dL; S:N, 18,008). cps. counts per second.

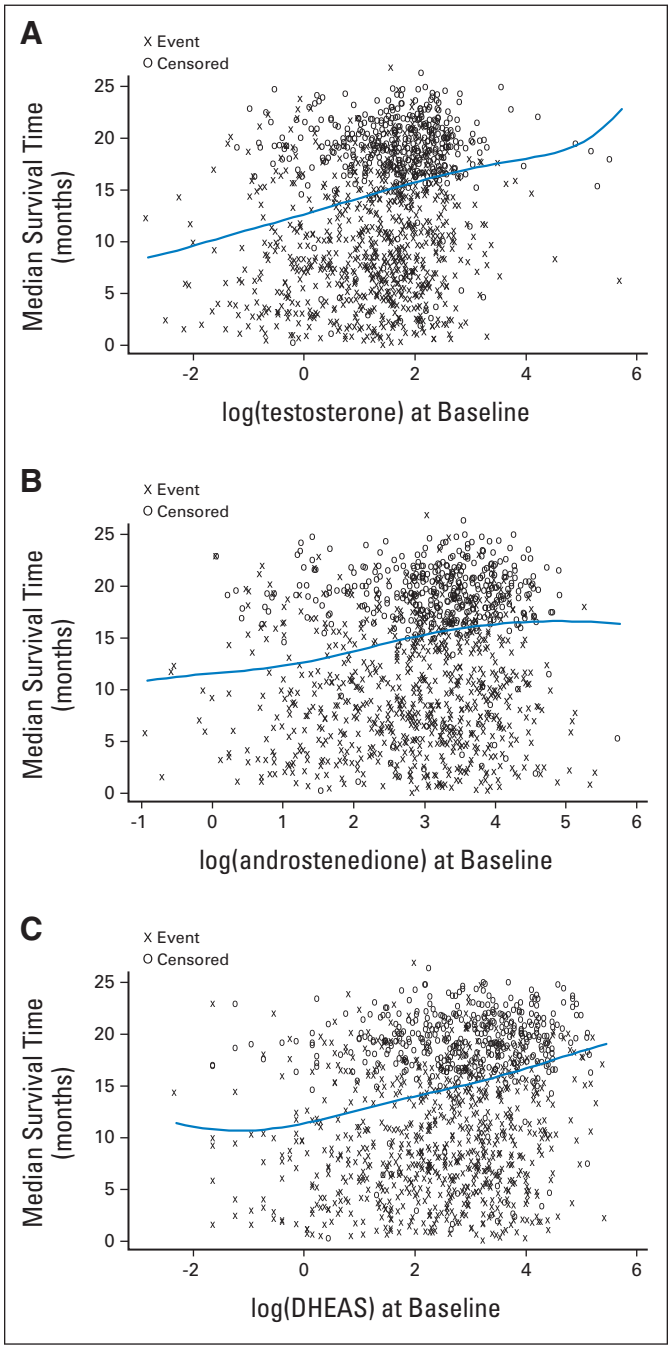

Fig A2. Relationship of distribution of baseline androgen levels and estimate of median survival in all patients. (A) Testosterone; (B) androstenedione; (C) dehydroepiandrosterone sulfate (DHEAS).