Effects of Abiraterone Acetate on Androgen Signaling in Castrate-Resistant Prostate Cancer in Bone

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See accompanying article on page 644; listen to the podcast by Dr Taplin at www.jco.org/podcasts

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ABSTRACT

Purpose

Persistent androgen signaling is implicated in castrate-resistant prostate cancer (CRPC) progression. This study aimed to evaluate androgen signaling in bone marrow-infiltrating cancer and testosterone in blood and bone marrow and to correlate with clinical observations.

Patients and Methods

This was an open-label, observational study of 57 patients with bone-metastatic CRPC who underwent transiliac bone marrow biopsy between October 2007 and March 2010. Patients received oral abiraterone acetate (1 g) once daily and prednisone (5 mg) twice daily. Androgen receptor (AR) and CYP17 expression were assessed by immunohistochemistry, testosterone concentration by mass spectrometry, AR copy number by polymerase chain reaction, and *TMPRSS2-ERG* status by fluorescent in situ hybridization in available tissues.

Results

Median overall survival was 555 days (95% CI, 440 to 965+ days). Maximal prostate-specific antigen decline $\geq 50\%$ occurred in 28 (50%) of 56 patients. Homogeneous, intense nuclear expression of AR, combined with $\geq 10\%$ CYP17 tumor expression, was correlated with longer time to treatment discontinuation (> 4 months) in 25 patients with tumor-infiltrated bone marrow samples. Pretreatment CYP17 tumor expression $\geq 10\%$ was correlated with increased bone marrow aspirate testosterone. Blood and bone marrow aspirate testosterone concentrations declined to less than picograms-per-milliliter levels and remained suppressed at progression.

Conclusion

The observed pretreatment androgen-signaling signature is consistent with persistent androgen signaling in CRPC bone metastases. This is the first evidence that abiraterone acetate achieves sustained suppression of testosterone in both blood and bone marrow aspirate to less than picograms-per-milliliter levels. Potential admixture of blood with bone marrow aspirate limits our ability to determine the origin of measured testosterone.

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INTRODUCTION

Recent clinical findings link persistent androgen signaling to castrate-resistant prostate cancer (CRPC) progression in some patients, leading to reevaluation of the widely held view that progression after castration is androgen independent.¹ Geller² framed this hypothesis more than 2 decades ago; more recently, Mohler et al³ and Titus et al⁴ provided findings that support the hypothesis that persistent androgen signaling is implicated in resistance to castration. Clinical findings of significant benefit, with occasional striking regression of CRPC and recently confirmed overall survival (OS) benefit after

CYP17 lyase inhibition or more specific androgen receptor (AR) blockade, establish the therapeutic relevance of Geller's hypothesis.⁵⁻¹² These findings have fostered interest in understanding the potential benefit of targeting AR signaling in the castrate-resistant state.

We performed a translational study treating patients with bone-metastatic CRPC with abiraterone acetate. We demonstrate that testosterone is depleted in blood and bone marrow aspirate to less than picograms-per-milliliter (pg/mL) levels after treatment and propose a candidate predictive signature based on findings in bone marrow-infiltrating prostate cancer cells.

PATIENTS AND METHODS

This open-label, single-center study was designed to evaluate androgen signaling in bone marrow-infiltrating cancer and testosterone in blood and bone marrow aspirate and to correlate findings with clinical observations. Secondary objectives included assessing treatment efficacy and safety and exploring the association between levels of circulating androgens and bone marrow aspirate androgens before and during treatment.

Patients had histologically confirmed adenocarcinoma of the prostate and castrate-resistant bone-metastatic disease progression. Disease progression was defined as documented osseous or soft-tissue metastatic progression or prostate-specific antigen (PSA) progression according to Prostate Cancer Clinical Trials Working Group II criteria (PCWG II).¹³ Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2 , a serum testosterone level ≤ 50 ng/dL (sustained by medical or surgical castration), normal serum potassium levels, and adequate renal, hepatic, and bone marrow function (Appendix, online only). All patients provided written informed consent, and the study protocol was approved by the MD Anderson Cancer Center institutional review board.

Treatment and Evaluation

Patients were treated with abiraterone acetate 1 g daily as four 250-mg tablets at least 1 hour before or 2 hours after a meal and prednisone 5-mg tablets twice daily. Screening and baseline evaluations included complete medical history, physical examination, complete blood count, serum electrolytes and chemistry, PSA levels, testosterone concentration, radionuclide bone scan, and tumor imaging (chest x-ray and pelvic and abdominal computed tomography scans). Safety assessments, using National Cancer Institute Common Terminology Criteria for Adverse Events version 3, were completed every 4 weeks, along with physical examination and select serum chemistry, electrolyte, PSA, and testosterone evaluations. Bone marrow biopsy and aspirate (~ 5 mL) were performed before treatment, at week 8, and at end of study. End-of-study biopsy was performed on treatment discontinuation. Matching blood plasma and serum were collected within 2 hours of biopsy.

Imaging studies were performed at the time of suspected prostate cancer progression or at the treating physician's discretion. Therapy was discontinued at the treating physician's discretion in patients exhibiting clinical progression, which was defined as worsening of preexisting, or development of new, disease-related symptoms.

Assay Methodologies

Tissue and derivatives banking and immunohistochemistry. The bone marrow specimens were obtained by transiliac biopsy, and samples were processed according to standard MD Anderson Cancer Center decalcification and fixation procedures. After pathologic evaluation, samples were stored in the MD Anderson Cancer Center Prostate Cancer Tissue Bank along with plasma from matching aspirate. Immunohistochemistry (IHC) was performed on 3.5-mm formalin-fixed, paraffin-embedded bone marrow biopsy sections for AR (dilution, 1:50; Dako, Carpinteria, CA) and CYP17 (dilution, 1:450; Novus, Littleton, CO). The AR antibody was a standard diagnostic antibody validated for clinical use, and we used accepted quality control measures. The CYP17 antibody IHC was validated by Western blots combined with IHC and was performed on appropriate positive controls (ie, ovarian lysates) and multitissue controls. A Dako autostainer and standard 3,3'-diaminobenzidine were used, as previously described.¹⁴ Marker expression was assessed by scoring two fields containing at least 100 tumor cells per specimen and expressed as a percentage. The involvement of cells exhibiting detectable staining was scored as 0 (no staining), 1 (up to 25%), 2 (25% to 75%), or 3 ($> 75\%$). The intensity of staining was scored as zero (no staining), low, or high. Subcellular distribution of biomarker expression was also recorded (membranous, cytosolic, nuclear, or combination).

AR copy numbers. Details of AR copy number methods are shown in the Appendix (online only).

TMPRSS2-ERG gene rearrangement status. Details of *TMPRSS2-ERG* gene rearrangement status assessment are shown in the Appendix (online only).

Mass spectrometry. Liquid chromatography-tandem mass spectrometry analysis of androgens was performed, as previously described (Appendix, online only).⁴

Statistical Considerations

The enrollment of approximately 60 patients would provide a data set of 30 paired bone marrow aspirates for evaluation of primary end points, with a 50% anticipated success rate for obtaining adequate bone marrow biopsies and aspirates. A sample size of 30 paired bone marrow aspirates (baseline and week 8) would provide 82% power to detect an effect of at least 0.55 in bone marrow testosterone levels using a two-sided paired *t* test at a .05 significance level. OS and time to treatment discontinuation from date of treatment initiation were estimated using the Kaplan-Meier method. Correlations between serum and bone marrow testosterone by mass spectrometry were assessed using Pearson's and Spearman's methods. The Wilcoxon signed-rank test was used to assess treatment duration between samples with and without CYP17 expression and AR overexpression and bone marrow testosterone levels between samples with and without CYP17 expression.

RESULTS

Clinical Outcomes

Table 1 summarizes the demographic, clinical, and tumor characteristics of 57 patients enrolled onto the study from October 2007 through March 2010. Of the patients, 57 were evaluable for safety and 56 for clinical response; 27 patients had tumor-infiltrated bone marrow at pretreatment (baseline), and 30 patients had tumor infiltration at any one time point. Median age was 70 years, and eight patients were older than 80 years. All patients had bone-metastatic CRPC. Most had received prior chemotherapy and several lines of hormonal manipulation (Table 1). Twenty patients (35%) had lymph node metastases, and eight patients (14%) had visceral metastases. Most patients had disease-related symptoms at study entry, and the median ECOG PS was 2 (range, 0 to 2).

To date, 49 of 57 patients have discontinued treatment, 30 with evidence of clinical progression, which was paired with imaging progression in 15 of 30 patients and with PSA progression (PCWG II) in six of 30 patients. Thirteen patients did not have clinical progression but discontinued treatment as a result of imaging and/or PSA progression (PCWG II). In addition, six patients discontinued treatment—two as a result of financial hardship, two per treating physician preference, one because of bowel obstruction attributed to radiation enteritis unrelated to study drug or disease, and one because of an ischemic cerebrovascular event unrelated to study drug or disease.

Patients received abiraterone acetate and prednisone treatment for a median of 233 days (7.6 months; range, 28 to 945+ days; 95% CI, 196 to 400 days; Fig 1). Therapy was well tolerated, with most adverse events categorized as grade 1/2 (National Cancer Institute Common Terminology Criteria for Adverse Events) and a safety profile consistent with other reported and anticipated effects of CYP17 inhibition with abiraterone acetate and systemic corticosteroids.⁵⁻¹² Seven grade 3 events were reported that were possibly related to study drug combination; these included elevated liver function tests ($n = 2$), hypokalemia ($n = 1$), hypertension ($n = 1$), and steroid-related hyperglycemia ($n = 3$).

A maximal PSA decline of more than 50% occurred in 28 (50%) of 56 evaluable patients, and nine patients (16%) had a more than 90% decline. PSA decline $\geq 30\%$ occurred in 34 (59%) of 56 patients (Fig 2).

Table 1. Patient (N = 57) and Tumor Characteristics

Characteristic	No.	%
Patients		
No. of evaluable patients, October 2007 to March 2010	56	
Race		
White	52	91
Black	3	5
Hispanic	2	4
Age, years		
Median	70	
Range	48-87	
Performance status		
Median	2	
Range	0-2	
Prior cytotoxic therapies		
Chemotherapy	48	84
≥ 2 regimens	34	60
Docetaxel-based regimens	45	79
Radiopharmaceuticals	7	12
Salvage hormonal therapies		
Ketoconazole	18	33
Estrogens and/or ketoconazole	36	65
Prior experimental treatments/novel agents	21	38
Time to CRPC from castration initiation, months		
Median	26	
Range	3-132	
Tumors		
PSA, ng/dL		
Median	95.3	
Range	3-2,725	
Extent of bone metastases		
< 10	10	16
≥ 10	47	82
> 20	39	68
Lymph nodes		
Local recurrence	20	35
Visceral metastases		
Liver	8	14
Lung	5	9
Adrenal	2	4
Bone marrow involvement detected by transiliac biopsy		
At baseline	30	53
At week 8	27	47
At week 8	18	32

Abbreviations: CRPC, castrate-resistant prostate cancer; PSA, prostate-specific antigen.

Fig 1. Kaplan-Meier plot of proportion of patients (y-axis) discontinuing treatment over time (x-axis). Figure illustrates two different types of disease progression occurring either soon after initiation (gold box) or after prolonged treatment.

disease course, and (2) the remainder of patients, who did experience effects (Fig 1). In patients following the first pattern, we observed no symptomatic improvement and no objective evidence of tumor regression; we considered these patients to exhibit primary resistance to treatment (14 [25%] of 56 patients). The remaining patients experienced longer periods of treatment and varied clinical response. ECOG PS improvement was self-reported in 25 (66%) of 38 initially symptomatic patients with delayed progression, whereas there was no patient-reported improvement in 12 symptomatic primary-resistant patients. Of note, the two initially asymptomatic patients in this group became rapidly symptomatic while on treatment. The two groups of patients did not differ significantly with regard to prior treatment or extent of disease at presentation.

Median OS for the entire cohort was 555 days (18.2 months; 95% CI, 440 to 965+ days; range, 49 to 1,037+ days).

Tissue and Aspirate Collection

Pretreatment bone marrow biopsies revealed tumor involvement in 27 (47%) of 56 patients, 25 (44%) of whom had ≥ 5% tumor infiltration evaluable for analysis (Table 1). Eleven of these were patients who discontinued treatment within 4 months of initiation. Bone marrow aspirates were collected pretreatment in 49 (88%) of 56 patients, at week 8 in 44 (84%) of 56 patients, and on treatment discontinuation in 27 (55%) of 49 patients. Six patients did not yield bone

Bone scans were performed in 36 patients after 6 months of treatment. Imaging improvement was recorded in three patients, stable disease in 26 patients, and more than two new lesions in seven patients. Of five patients with hepatic metastases, two were reevaluated by imaging; one was found to have achieved partial response, and one was found to have stable disease. One of three patients with lung metastases had a partial response. Among 10 patients with lymph node involvement reevaluated by imaging, five patients had evidence of a partial response, two had stable disease, and three had progressive disease.

Although the rate of progression seemed to be somewhat steady, with gradual decline over time through 200 days, we observed evidence of two different patterns of clinical outcome with abiraterone acetate: (1) patients who experienced no apparent effect on clinical

Fig 2. Waterfall plot representing the percentage changes in serum prostate-specific antigen (PSA) concentrations. Maximal PSA reduction is represented on the right. Dashed lines represent 25%, -30%, and -90% PSA change.

Table 2. Blood and Bone Marrow Aspirate Plasma Testosterone Concentrations Assessed by Mass Spectrometry

Androgen	Baseline		8 Weeks		End of Study*	
	Range (ng/mL)	No.	Range (ng/mL)	No.	Range (ng/mL)	No.
Testosterone						
Blood plasma	0.0000-0.214	52	0.0000†-0.0129	49	0.0000	32
Bone marrow aspirate plasma	0.0000-0.257	49	0.0000	44	0.0000	27
Dihydrotestosterone						
Blood plasma	0.0000-0.0459	52	0.0000-0.0203‡	49	0.0000	32
Bone marrow aspirate plasma	0.0000-0.257§	49	0.0000	44	0.0000	27

*End-of-study samples coinciding with week 8 not included.

†Only one patient had detectable testosterone.

‡Two patients had detectable week 8 dihydrotestosterone.

§Two patients had detectable pretreatment bone marrow aspirate dihydrotestosterone.

marrow aspirates for study at any time point (dry tap). Bone marrow aspirates from two or more time points were collected in 40 (71%) of 56 patients. Blood plasma and serum samples were collected pretreatment in 52 patients (93%), at week 8 in 49 patients (88%), and on treatment discontinuation in 43 (88%) of 49 patients.

Bone Marrow Aspirate and Blood Testosterone and Dihydrotestosterone Concentrations

Bone marrow aspirate and blood plasma testosterone and dihydrotestosterone (DHT) measurements by mass spectrometry confirmed metabolite depletion at 8 weeks of treatment and at end of study (on treatment discontinuation) (Table 2; see also Appendix Fig A1, online only). Additional random measurements of circulating testosterone levels at different time points before discontinuation confirmed testosterone depletion (41 samples, 15 patients with prolonged treatment). In the subset of patients with both pretreatment plasma and bone marrow aspirate study specimens available for analysis, a strong correlation (Pearson's $r = 0.91$) between pretreatment circulating and microenvironment bone marrow aspirate testosterone levels was found by mass spectrometry (Appendix Fig A1). Bone marrow aspirate plasma testosterone concentration was higher than circulating blood plasma testosterone in seven of 42 available cases with matching evaluable samples. Pretreatment bone marrow aspirate DHT was undetectable in the bone marrow aspirate in 46 of 48 measured samples. In 52 samples, mean blood DHT was 0.0116 ng/mL, with a range

of 0.0000 to 0.0459 ng/mL. It was undetectable in 27 of 52 plasma samples. No correlation was seen between blood and bone marrow DHT, but this could be a result of the undetectable levels of DHT ($< \text{pg/mL}$). DHT was not detected in any bone marrow aspirate after treatment, whereas it remained detectable in only two of 49 blood 8-week treatment samples; of note, testosterone was undetectable in those samples. Bone marrow aspirate and blood DHT were undetectable on treatment discontinuation (Table 2).

Molecular Characterization of Bone Marrow Metastases

Nuclear AR expression varied in involvement and intensity within and among samples in pretreatment bone marrow biopsies (Table 3). AR expression ranged from 2+ to 3 (50% to 100% involvement) and was of moderate to high intensity. Cytoplasmic CYP17 expression in tumor cells was heterogeneous in involvement and intensity and ranged from 0 to 2+. Homogeneous, intense expression of nuclear AR (involvement ≥ 3 , high intensity) in combination with 1+ ($\geq 10\%$) CYP17 tumor expression was correlated with longer time to treatment discontinuation (> 4 months) in the 25 available samples ($P < .001$, Wilcoxon signed-rank test; Fig 3A, Table 3). Pretreatment CYP17 tumor expression was correlated with increased bone marrow aspirate plasma testosterone concentration ($P = .045$) in 19 evaluable paired samples (Fig 3B).

Table 3. Pretreatment Nuclear AR and Cytoplasmic CYP17 Expression and Correlation of Increased Nuclear AR Expression and Cytoplasmic CYP17 Expression With Treatment Duration

Expression With Treatment Duration				
Pretreatment Marker Expression	0 (No Expression)	1 (1%-25%)	2 (26%-75%)	3 (> 75%)
Nuclear AR, No.	0	0	8	17
Cytoplasmic CYP17, No.	4	5	8	8
Outcome	Primary Resistance (treatment ≤ 4 months)	Longer Treatment Duration (treatment > 4 months)	<i>P</i> (Wilcoxon signed-rank test)	
Pretreatment nuclear AR expression 3+ and CYP17 expression in tumor epithelium				
No.	1	12	< .001	
%	7	82		
Lack of one or both				
No.	10	2		
%	91	18		

Abbreviation: AR, androgen receptor.

Fig 3. (A) Pretreatment intense nuclear androgen receptor (AR) expression in combination with CYP17 expression in the bone marrow–infiltrating tumor of a patient with treatment duration more than 4 months. (B) Pretreatment CYP17 expression in tumor is correlated with increased bone marrow aspirate (BMA) plasma testosterone (T) concentration in 19 cases with paired samples. MS, mass spectrometry.

TMPRSS2-ERG gene rearrangement status and AR copy number were evaluable in 15 of 25 patients with $\geq 20\%$ tumor involvement. *TMPRSS2-ERG* rearrangement was identified in three of 15 evaluable samples. Pretreatment AR copy numbers were low. Six patients had evaluable serial specimens for analyses. Four patients with longer treatment duration displayed AR copy number increase during treatment, whereas no change was seen in two patients who discontinued therapy because of progression after 8 weeks of treatment.

DISCUSSION

Prostate cancer progression that invariably follows the initial benefit from androgen ablation has, until recently, been considered androgen independent. In addition, the residual concentration of androgens

observed after androgen ablation was considered inconsequential, and progression of CRPC was attributed to androgen-independent mechanisms by most investigators. However, recent results support the view that persistent androgen signaling in the castrate-resistant state is functionally significant and a validated therapeutic target in CRPC.^{2,11,12,15,16} Similarly, the efficacy of this androgen biosynthesis inhibitor recently approved by the US Food and Drug Administration establishes the importance of persistent androgen signaling in CRPC progression.

A $\geq 50\%$ maximal PSA decline occurred in 28 (50%) of 56 patients in the study population despite the extensive cancer involvement in patients selected for this study. The favorable safety profile observed was similar to that reported by others and recently confirmed in a phase III study.⁵⁻¹² Some patients experienced fatigue and weakness, but we could not distinguish these from the adverse effects of progressive prostate cancer.

For the purpose of this study, patients were divided into two categories defined by duration of therapy: those who had undergone ≤ 4 months of therapy and the remainder. The 14 patients (25%) who were treated for ≤ 4 months continued to have worsening of cancer-related symptoms, with no findings to suggest benefit from abiraterone acetate and prednisone treatment, and were considered to have exhibited primary resistance to abiraterone acetate. In contrast, patients who were treated with therapy of longer duration in general experienced improvement in symptoms and had findings consistent with antitumor activity. The present results will not determine the point in progression when resistance to abiraterone acetate emerges. Understanding the relationship between the emergence of resistance and progression will guide further development of therapy.¹⁷

This study establishes the feasibility and value of sampling bone marrow biopsies in selected patients to gain insight into the mechanism of resistance to molecularly targeted therapies in human prostate cancer, following the initial bone biopsy experiment reported by Taplin et al.¹⁸ Accordingly, uniform and intense tumor nuclear AR expression, coupled with cytoplasmic CYP17 expression, were linked to lack of primary resistance to abiraterone acetate. We also observed a correlation between pretreatment bone marrow aspirate testosterone levels and tumor CYP17 expression.

These associations are consistent with the hypothesis that patients with CRPC demonstrate persistent AR signaling in the tumor microenvironment and are more likely to benefit from therapy with androgen biosynthesis inhibitors. The correlation of pretreatment testosterone levels in blood and bone marrow to CYP17 expression supports the hypothesis that CYP17 expression is functionally meaningful. We have preclinical and clinical tissue-based data in support of increased tumor CYP17 expression after standard androgen ablation strategies as further indirect support of enzyme functionality (data not shown).

As previously reported, abiraterone acetate effectively depletes testosterone in the blood of patients with CRPC.⁵ We have extended these observations, and to our knowledge, we are the first to demonstrate that abiraterone acetate depletes blood and bone marrow aspirate testosterone and DHT concentrations to less than pg/mL levels and that this depletion is sustained at treatment discontinuation. The potential for blood admixture with bone marrow aspirate limits the ability to determine the site of production of the measured testosterone in bone marrow aspirate. The progression observed in the presence of a depleted testosterone environment leads us

to propose that native ligand-independent mechanisms are likely to drive progression during treatment with androgen biosynthesis inhibitors. Our results do not exclude the possibility that altered steroid biosynthesis in the tumor microenvironment accounts for progression in a testosterone-depleted environment. The predominant enzyme that irreversibly converts testosterone to DHT, steroid 5 α -reductase type 2, exhibits decreased gene and protein expression in prostate cancer cells during progression of prostate cancer to advanced prostate cancer.^{4,19,20} Investigators have speculated, though, that a feedback mechanism, such as increased “backdoor” biosynthesis of DHT,²¹ may act as an alternative ligand-dependent mechanism of resistance to abiraterone acetate; the present DHT results do not support this hypothesis. A shortcoming of our approach was the inability to assess cellular androgen levels given the limited tissue collected from bone. Although the present results establish the feasibility of sampling bone marrow biopsies, critical concerns remain. The potential mixture of blood with bone marrow supernatant does not permit us to conclude that the bone marrow supernatant–detected testosterone is of tumor microenvironment origin. The low level of bone marrow aspirate DHT compared with circulating DHT in 25 of 52 patients, which is consistent with lower levels of metabolite anticipated in metastatic sites, and the correlation of CYP17 expression with bone marrow aspirate testosterone concentration suggests that microenvironment production contributes to measured testosterone in bone marrow aspirate. Although these data are supportive of therapeutically relevant production of steroid hormones, further refinement of techniques to account for blood admixture will be required to provide more definitive conclusions. The study afforded us the opportunity to explore the feasibility of assessing AR copy number and *TMPRSS2-ERG* in bone marrow biopsy specimens; however, too few specimens were available to assign significance to the observations.

Our findings support the view that persistent androgen signaling is implicated in the progression of CRPC and can be targeted therapeutically. Despite the small sample size, the present data imply a role for AR signaling in disease progression. The observa-

tions we report form the basis for the development of a predictive signature that may be used to select patients for treatment with abiraterone acetate.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(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.

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