Improvements in Radiographic Progression-Free Survival Stratified by ERG Gene Status in Metastatic Castration-Resistant Prostate Cancer Patients Treated with Abiraterone Acetate

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Abstract

Purpose: Gene fusions leading to androgen receptor–modulated ERG overexpression occur in up to 70% of metastatic castration-resistant prostate cancers (mCRPC). We assessed the association between ERG rearrangement and clinical benefit from abiraterone acetate.

Experimental Design: COU-AA-302 is a phase III trial comparing abiraterone acetate and prednisone versus prednisone in the COU-AA-302 trial. However, our data suggest that 2+ Edel cancers, accounting for 15% of all mCRPC patients and previously associated with a worse outcome, derived the greatest benefit.

Results: ERG status was defined for 348 of 1,088 intention-to-treat patients. ERG was rearranged in 121 of 348 patients with confirmed ERG status (35%). Cancers with an ERG fusion secondary to deletion of 21q22 and increased copy number of fusion sequences (class 2+ Edel) had a greater improvement in rPFS after abiraterone acetate and prednisone [22 vs. 5.4 months; HR (95% confidence interval), 0.31 (0.15–0.68); P = 0.0033] than cancers with no ERG fusion [16.7 vs. 8.3 months; 0.53 (0.38–0.74); P = 0.0002] or other classes of ERG rearrangement. There was also greater benefit in this subgroup for TTPP.

Conclusions: Both ERG-rearranged and wild-type cancers had a significant improvement in rPFS with abiraterone acetate and prednisone in the COU-AA-302 trial. However, our data suggest that 2+ Edel cancers, accounting for 15% of all mCRPC patients and previously associated with a worse outcome, derived the greatest benefit.

Introduction

Abiraterone acetate is a prodrug of abiraterone, a selective androgen biosynthesis inhibitor that blocks cytochrome P450 17A1 (CYP17A1) and suppresses androgen and estrogen synthesis (1, 2). Used in combination with prednisone, abiraterone acetate is now approved for use in men with metastatic castration-resistant prostate cancer (mCRPC) in both the pre- and post-chemotherapy-treated settings based on demonstrated survival benefit. A review of the outcomes in these populations shows that the response in individual patients ranged from prolonged and durable to none at all, suggesting the presence of molecular alterations in tumors that predict for response. This finding, coupled with the recent approval of several other effective treatments for mCRPC, has highlighted the urgent need for predictive biomarkers that enrich for patient subpopulations in which treatment has a significant effect on clinically meaningful benefits. These associations are best demonstrated in the setting of randomized clinical trials.

Overexpression of E26 transformation–specific (ETS) transcription factor family members has been implicated in prostate cancer progression (3). Recurrent ETS gene fusions have been shown to occur in 50% to 70% of treatment-naïve prostate cancers, resulting in androgen-driven overexpression of ETS family members, most
Translational Relevance

Abiraterone acetate improves survival in metastatic castration-resistant prostate cancer (mCRPC), but not all patients respond. With the recent approval of several effective treatments for CRPC, there is an urgent need to develop biomarker strategies that identify patient subgroups enriched for sensitivity to a specific treatment. Here we demonstrate that although all ERG classes demonstrated an improvement in radiographic progression-free survival with abiraterone acetate and prednisone compared with prednisone alone in the COU-AA-302 phase III trial, the molecular subclass 2þ Edel, which was previously shown to be associated with worse survival, appeared to derive the greatest benefit. Although the biologic explanation for this observation remains unclear, our data introduce the possibility that specific biologic subtypes of mCRPC are more sensitive to androgen receptor targeting with abiraterone acetate. This supports the further evaluation of multiplex biomarker panels that include 2þ Edel ERG rearrangement status defined by FISH studies.

commonly ERG on 21q22.2 (4, 5). We therefore hypothesized that ERG-rearranged prostate cancers represent a molecular subtype of prostate cancer that is more sensitive to endocrine manipulations targeting androgen-driven signaling. Examination of patient-matched archival therapy-naïve prostate cancer tissue, CRPC tumor biopsies, and circulating tumor cells obtained before starting abiraterone acetate revealed that genomic ERG rearrangement status did not change with the development of castration resistance (6). This supported the evaluation of ERG gene fusions in archival samples as a predictive biomarker for patients with mCRPC receiving treatment with abiraterone acetate and prednisone.

We previously demonstrated a significant association between ERG rearrangement identified by FISH and magnitude of PSA decline in a cohort of pre- and post-chemotherapy patients with CRPC treated with abiraterone acetate in phase I/II trials (6). ERG gene fusions can occur following either deletion or rearrangement of the region 5′ of ERG, and manifest using a break-apart FISH assay with probes to ERG and 5′ of ERG as either loss of the 5′ probe or a split signal (Supplementary Fig. S1). These 2 classes are hereafter referred to as Edel and Esplit, respectively. The Edel class can be further subdivided into 1 Edel and 2þ Edel, characterized in the latter case by more than one ERG gene fusion sequence. Cancers with 2þ Edel ERG rearrangements are associated with worse clinical outcomes independent of the effect of aneuploidy (7, 8). ERG gene fusions can also be identified using PCR-based assays, but no association was observed between the presence of TMPRSS2: ERG fusion transcripts and PSA decline following treatment with abiraterone acetate (9).

COU-AA-302 was a phase III randomized, double-blind, placebo-controlled study comparing the efficacy and safety of abiraterone acetate and prednisone versus prednisone alone in asymptomatic or mildly symptomatic chemotherapy-naïve patients with mCRPC. Abiraterone acetate and prednisone significantly improved radiographic progression-free survival (rPFS) and demonstrated a trend toward improved overall survival (OS), leading to extension of the regulatory approval for abiraterone acetate to include patients who had not received prior chemotherapy. Patients treated with abiraterone acetate and prednisone also showed a significant improvement in the prespecified secondary end points, including declines in PSA and time to PSA progression (TTP; refs. 10, 11).

This prospectively defined biomarker substudy of COU-AA-302 aimed to evaluate the association between ERG rearrangement subclasses defined by break-apart FISH and clinical outcome in patients with chemotherapy-naïve mCRPC receiving abiraterone acetate and prednisone. We focused on ERG because it accounts for 90–95% of hormone-driven ETS family member prostate cancer gene rearrangements (4).

Materials and Methods

Study design and treatment

The evaluation of association of TMPRSS2: ERG tumor status with clinical outcome after treatment with abiraterone acetate and prednisone was prospectively defined as an exploratory end point in the COU-AA-302 trial. A total of 1,088 patients were included in the trial as described previously (abiraterone acetate and prednisone, 546; prednisone alone, 542). Briefly, patients were required to have metastatic, histologically, or cytologically confirmed prostate cancer, PSA progression according to Prostate Cancer Clinical Trials Working Group 2 criteria or radiographic progression in soft tissue or bone with or without PSA progression, and ongoing androgen deprivation with a serum testosterone level of less than 50 ng/dL (1.7 nmol/L; ref. 11). Patients with visceral disease were excluded. Patients were stratified by Eastern Cooperative Oncology Group (ECOG) performance status (PS; 0 vs. 1) and randomized 1:1 to abiraterone acetate 1 g daily and prednisone 5 mg twice daily or placebo and prednisone 5 mg twice daily. The co-primary end points were rPFS by independent review and OS. rPFS was defined as the time from randomization to the first occurrence of either progression by bone scan, progression by CT or MRI as defined by modified RECIST version 1.0 criteria or death from any cause. rPFS by investigator review of bone scans was also conducted. Prespecified secondary end points included time to rPFS by investigator review, TTP based on Prostate Cancer Working Group 2 criteria (12), and PSA response ≥50% PSA decline from baseline). Patients were offered the option to give informed consent to evaluation of TMPRSS2: ERG status in their tumor samples, and participating centers collected all available archival samples, which were then shipped to a central laboratory. Approval was obtained from the institutional review board of the participating centers.

ERG gene status was assessed in primary tumor samples by FISH as described previously (7); the assessment was conducted by ORIDIS Biomarkers. We used archived, paraffin-embedded, formalin-fixed pathology slides from (i) diagnostic tissue biopsy, (ii) radical prostatectomy specimens, (iii) transurethral resection of the prostate (TURP) specimens, or (iv) tumor bone marrow, lymph nodes, or other metastatic sites. Characterization of ERG classes is shown in Supplementary Fig. S1. Classification of ERG gene status prioritized 2þ Edel over 1 Edel such that if regions of 2þ Edel were observed in combination with other classes, the patient was classified as 2þ Edel. Similarly, 1 Edel was prioritized over Esplit. A 1% cutoff was used for all ERG patterns, calculated on the basis of analyses of normal epithelium as described.
previously (13). Clinical outcomes assessed for association with ERG status were rPFS, TTPP, and PSA response (≥50% decline from baseline).

Statistical analyses
Distributions of time-to-event variables with 2-sided 95% confidence intervals (CI) were estimated using the Kaplan–Meier product limit method. The stratified log-rank test was used for comparisons of time-to-event outcomes for abiraterone acetate and prednisone and prednisone alone subgroups according to ERG rearrangement status. Cox regression was used to evaluate association of ERG status through HR with ORs with Cochran–Mantel–Haenszel for PSA response in each treatment group separately and in the overall population. Analysis was performed at 43% or 56% OS events as specified in the text. Subjects with missing data for ERG status were excluded from the association analysis. If a subject had missing data for a clinically defined end point, that subject was excluded from that particular association analysis. All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc.).

Results
Sample collection and patient characteristics
Of the 1,088 total patients in the intention-to-treat population of the COU-AA-302 trial, 826 consented to participate in this biomarker substudy and samples from 501 patients were collected, including 2 samples from 14 patients and 3 or more samples from 4 patients (Fig. 1). The trial was conducted at 151 sites, of which 119 contributed at least one sample. The majority of patient tissue samples were from transrectal biopsies of the prostate (n = 357), with other tissue samples obtained from radical prostatectomy (n = 113), TURP (n = 44), bone marrow (n = 3), lymph nodes (n = 6), and other metastatic sites (n = 7). ERG rearrangement class was defined for 348 of 501 (69%) patients (abiraterone acetate and prednisone, n = 178; prednisone alone, n = 170). Unusable samples (n = 171) resulted from various technical issues, including missing sample (n = 35), tissue lost in processing (n = 36), lack of a hybridization signal (n = 69), background binding of probe (n = 3), and autofluorescence (n = 28).

Demographics and baseline characteristics of the 348 patients with confirmed ERG status were similar to those of the intention-to-treat population, with the following exceptions: median time from initial diagnosis and time from start of luteinizing hormone–releasing hormone analog to first dose of abiraterone acetate and prednisone were shorter and more patients were treated by surgery in the biomarker population (Table 1). These differences may be reflective of a higher success rate for obtaining tumor samples from more recently diagnosed patients and those treated by surgery.

Distribution of ERG rearrangement classes
ERG was rearranged in 121 of 348 patients (35%); abiraterone acetate and prednisone, n = 112; prednisone alone, n = 115). Of the 348 patients with confirmed ERG status, 6 patients with an ERG rearrangement had solely an Esplit (2%) and 115 patients...
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Table 1. Demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA + P (n = 178)</td>
<td>P (n = 170)</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>70 (49–90)</td>
</tr>
<tr>
<td>ECOG PS 1</td>
<td>21%</td>
</tr>
<tr>
<td>Gleason ≥ 8</td>
<td>56%</td>
</tr>
<tr>
<td>Median testosterone, ng/mL (range)</td>
<td>0.4 (0.1–2.2)</td>
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<tr>
<td>Median LDH, IU/L (range)</td>
<td>181 (60–871)</td>
</tr>
<tr>
<td>Median ALP, IU/L (range)</td>
<td>92 (32–1,297)</td>
</tr>
<tr>
<td>Median Hb, g/dL (range)</td>
<td>13.2 (10–15.5)</td>
</tr>
<tr>
<td>Median PSA, ng/mL (range)</td>
<td>38 (2–1,716)</td>
</tr>
<tr>
<td>Median time from initial diagnosis to first dose, y</td>
<td>4.3</td>
</tr>
<tr>
<td>Previous cancer treatment</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>51%</td>
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<tr>
<td>Radiotherapy</td>
<td>51%</td>
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<tr>
<td>Bone</td>
<td>80%</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>47%</td>
</tr>
<tr>
<td>Other</td>
<td>1%</td>
</tr>
</tbody>
</table>

Abbreviations: AA, abiraterone acetate; ALP, alkaline phosphatase; Hb, hemoglobin; ITT, intention to treat; LDH, lactate dehydrogenase; LHRHa, luteinizing hormone–releasing hormone analog; P, prednisone.

(33%) had an Edel-type rearrangement. Sixty-four patients with an Edel-type rearrangement were of the 1 Edel class (18%) and 51 (15%) were of the 2 Edel class (Table 2). Fourteen 1 Edel cancers had areas with Esplit and 45 2 Edel cancers had areas with 1 Edel and were prioritized as 1 Edel or 2 Edel cancers, respectively. Because there were too few Esplit cancers, this class was not included in the analyses of association with clinical outcome. Demographic and baseline characteristics were well-balanced between the rearranged and nonrearranged classes (Supplementary Table S1).

Improvements in rPFS stratified by ERG class

A significant improvement in rPFS, as measured by independent radiographic review, with abiraterone acetate and prednisone versus prednisone alone was observed in the biomarker population, as described previously in the intention-to-treat population (ref. 10; Fig. 2). Treatment with abiraterone acetate plus prednisone resulted in an improvement in rPFS relative to prednisone alone for all classes of ERG rearrangement. By category, the differences were 22.0 versus 5.4 months for 2 Edel cancers [HR (95% CI): 0.31 (0.15–0.68); P = 0.0033; Fig. 3A], 13.8 versus 10.9 months for 1 Edel class [HR (95% CI): 0.56 (0.29–1.08); P = 0.0852; Fig. 3B], and 16.4 versus 8.3 months nonrearranged class [HR (95% CI): 0.53 (0.38–0.74); P = 0.0002; Fig. 3C]. These data suggest that the improvement in rPFS with abiraterone acetate and prednisone for 2 Edel versus nonrearranged cancers [HR (95% CI), 0.58 (0.30–1.09); P = 0.05] is greater than the difference in improvement between other ERG classes [HR (95% CI), 0.96 (0.58–1.61), P = 0.89; Fig. 3D]. There was no difference in rPFS as assessed by investigator review or OS and different ERG classes (Supplementary Table S2).

TTPP stratified by ERG class

The TTPP in 2 Edel cancers treated with abiraterone acetate and prednisone was 14 months compared with 8.3 months in 1 Edel and 8.4 months in nonrearranged cancers (Fig. 4A). As with rPFS, the improvement in TTPP in the abiraterone acetate and prednisone arm in 2 Edel compared with nonrearranged cancers [HR (95% CI), 0.63 (0.38–1.05); P = 0.0784] was greater than in 1 Edel versus nonrearranged cancers. Similarly, there was no difference in TTPP between different ERG classes in the prednisone alone arm (Fig. 4B). There was no difference in magnitude of PSA decline, which is a less clinically relevant end point, between different ERG classes (Supplementary Table S3).

Discussion

Patients with chemotherapy-naive mCRPC derive greater clinical benefit from abiraterone acetate and prednisone compared with prednisone alone, independent of ERG status. The improvement in rPFS and TTPP was greatest in patients with 2 Edel cancers but did not reach statistical significance, likely due to the small sample size. This interim analysis of the COU-AA-302 trial did not reach significance for OS, and therefore, it was not surprising that we did not observe a significant association between ERG status and OS. However, we previously demonstrated a robust association between rPFS and OS (14), which provided support for the use of rPFS as a primary/co-primary endpoint in the context of improved TTPP and OS.
endpoint in phase III mCRPC studies. In this biomarker population, investigator-defined rPFS was sufficiently different and overall less accurate than centrally reviewed rPFS to explain the absence of the association observed for central rPFS. Noteworthy was that 2+ Edel cancers are associated with a significantly worse outcome in patients who never received abiraterone acetate (7, 8). The 15% frequency of 2+ Edel cancers among patients in the COU-AA-302 population with confirmed ERG status is higher than observed previously at the time of diagnosis (7%; ref. 7). Nonetheless, the small overall number of cases (51 in total) limited our ability to confirm a statistically significant difference in HR for the different ERG classes.

The biologic explanation for these observations is unclear. It is possible that 2+ Edel cancers have significantly higher levels of expression of androgen-driven ERG protein as a result of fusion sequence copy number gain and are therefore more sensitive to downregulation of ERG following inhibition of androgen receptor activation by abiraterone acetate. Alternatively, 2+ Edel cancers could represent a molecularly distinct subclass with structural differences additional to duplication of ERG gene fusion sequences. Ongoing molecular studies could explain the differential outcome of ERG fusion subclasses. Although studies on metastases obtained at rapid autopsy or by biopsy or capture of circulating tumor cells reported consistent 21q deletion in all CRPC metastases (6, 15), emerging evidence from circulating tumor DNA studies suggests the presence in CRPC of distinct clones with different ERG status from the primary tumor (16) This could suggest that strategies for tracking ERG status in multiple heterogeneous CRPC clones at initiation of abiraterone acetate could more accurately confirm an association between class 2+ Edel and outcome.

Baseline serum androgens (17), circulating tumor cell count (CellSearch; Scher and colleagues, unpublished observations, 2014) and neutrophil/lymphocyte ratio (18) have been shown...
to provide prognostic information in patients receiving treatment with abiraterone acetate and prednisone. Also required are biomarkers that identify patients who are most likely to respond in order to guide treatment decisions and improve the prostate cancer management paradigm. The COU-AA-302 phase III study succeeded in collecting tumor samples from close to half of all accrued patients. This confirms the enthusiasm in the prostate cancer community to support molecular characterization analyses, and we trust this will encourage tissue collection to be similarly integrated into future phase III CRPC trials.

There is currently no prospectively collected set of samples that could allow further evaluation of the observations in this study. Overall, our data suggest that identification of 2+ Edel cancers could have a role in a predictive biomarker panel for identifying patients with mCRPC who may be more likely to respond to abiraterone acetate and prednisone. Strategies that recognize the potential emergence of CRPC clones with a different ERG status and additional factors that could refine the prediction further are under evaluation.

Disclosure of Potential Conflicts of Interest

G. Attard reports receiving a commercial research grant from AstraZeneca and Janssen; speakers bureau honoraria from Astellas, Janssen, Roche/Ventana, Sanofi-Aventis, and Takeda; has ownership interest (including patents) in Institute of Cancer Research: Rewards to Inventors; is a consultant/advisory board member for Abbott Laboratories, Astellas, Janssen, Medication, Millenium Pharma, Novartis, Roche/Ventana, and Veridea; and is included in the co-inventors reward scheme for abiraterone acetate, which was developed at The Institute of Cancer Research and is now licensed to Janssen Biotech. J.S. de Bono reports receiving a commercial research grant from and is a consultant/advisory board member for Astellas and Janssen. A.M. Joshua reports receiving a commercial research grant from and is a consultant/advisory board member for Astellas, Bristol-Myers Squibb, Excelixsis, Johnson and Johnson, Novartis, and Pfizer. K. Fizazi and C.J. Ryan report receiving page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Figure 4.

Time to PSA progression* by ERG class in the abiraterone acetate and prednisone arm (A) or prednisone alone arm (B). *Analysis at 56% overall survival events.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G. Attard, J.S. de Bono, C.J. Logothetis, S.D. Mukherjee, W. Li, A. Molina, T.W. Griffin


Other (final approval of manuscript): G. Attard

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