Resistance to CYP17A1 Inhibition with Abiraterone in Castration-Resistant Prostate Cancer: Induction of Steroidogenesis and Androgen Receptor Splice Variants

Elahe A. Mostaghel1,3, Brett T. Marck4, Stephen R. Plymate3,4, Robert L. Vessella5, Stephen Balk6, Alvin M. Matsumoto3,4, Peter S. Nelson1,2,3, and R. Bruce Montgomery3,4

Abstract

Purpose: Abiraterone is a potent inhibitor of the steroidogenic enzyme CYP17A1 and suppresses tumor growth in patients with castration-resistant prostate cancer (CRPC). The effectiveness of abiraterone in reducing tumor androgens is not known, nor have mechanisms contributing to abiraterone resistance been established.

Experimental Design: We treated human CRPC xenografts with abiraterone and measured tumor growth, tissue androgens, androgen receptor (AR) levels, and steroidogenic gene expression versus controls.

Results: Abiraterone suppressed serum PSA levels and improved survival in two distinct CRPC xenografts: median survival of LuCaP35CR improved from 17 to 39 days (HR = 3.6, P = 0.0014) and LuCaP23CR from 14 to 24 days (HR = 2.5, P = 0.0048). Abiraterone strongly suppressed tumor androgens, with testosterone (T) decreasing from 0.49 ± 0.22 to 0.03 ± 0.01 pg/mg (P < 0.0001), and from 0.69 ± 0.36 to 0.03 ± 0.01 pg/mg (P = 0.002) in abiraterone-treated 23CR and 35CR, respectively, with comparable decreases in tissue DHT. Treatment was associated with increased expression of full-length AR (ARFL) and truncated AR variants (ARFL 2.3-fold, P = 0.008 and ARdel567es 2.7-fold, P = 0.036 in 23 CR; ARFL 3.4-fold, P = 0.001 and ARV7 3.1-fold, P = 0.0003 in 35CR), and increased expression of the abiraterone target CYP17A1 (~2.1-fold, P = 0.0001 and P = 0.028 in 23CR and 35CR, respectively) and transcript changes in other enzymes modulating steroid metabolism.

Conclusions: These studies indicate that abiraterone reduces CRPC growth via suppression of intratumoral androgens and that resistance to abiraterone may occur through mechanisms that include upregulation of CYP17A1, and/or induction of AR and AR splice variants that confer ligand-independent AR transactivation.

Clin Cancer Res; 17(18); 5913–25. ©2011 AACR.

Introduction

Prostate cancer develops resistance to serum androgen suppression in essentially all patients with advanced disease (1). Upregulated androgen receptor (AR) expression and autonomous synthesis of androgens by neoplastic prostate epithelium (either de novo from cholesterol or through metabolism of adrenal precursors) are important contributors to castration-resistant prostate cancer (CRPC) growth (2–5). Tissue androgens such as dihydrotestosterone (DHT) may be maintained via the "classical" pathway of steroidogenesis proceeding through dehydroepiandrosterone (DHEA), or through a "back-door" pathway using 5α-reduced steroid precursors as the primary source of DHT (6). In addition to upregulated expression of full-length AR (ARFL), the generation of constitutively active AR variants by differential transcript splicing of the ligand-binding domain has been described (2, 7–12). These AR splice variants have ligand-independent activity, and ARV7 can also function by enhancing the response of ARFL to low ligand concentrations (10, 13).

Abiraterone is a novel agent designed to suppress growth of CRPC by inhibiting CYP17A1, a rate limiting enzyme of steroidogenesis (14). Potential sites of action include any organ capable of elaborating androgens, including testis, adrenal gland, or prostate cancer tissue. Positive results in...
**Translational Relevance**

Abiraterone is a novel CYP17A1 inhibitor recently shown to extend the survival of men with castration-resistant prostate cancer (CRPC). However, many men ultimately progress and mechanisms of resistance to abiraterone treatment in vivo have not been elucidated. We show in two xenograft models of CRPC that the clinical response to abiraterone is accompanied by marked suppression of tumor androgen levels, and identify increased expression of AR ligand-independent AR splice variants, and the steroidogenic transcriptome (including the CYP17A1 target gene) as potential mechanisms of adaptation to CYP17A1 blockade. These data suggest that resistance can potentially be targeted by using higher dose levels of abiraterone or combinations with potent AR antagonists currently in development and show that more effective suppression of AR signaling remains an important means of effectively treating advanced prostate cancer.

Materials and Methods

LuCaP human prostate cancer xenografts

The establishment and maintenance of the LuCaP23 and 35 xenografts from lymph node metastases of 2 individuals with CRPC as a component of the University of Washington Rapid Autopsy program has been previously described (21, 22). The AR has been sequenced and codes for a wild-type protein in both xenografts. In eugonadal hosts, these lines produce serum PSA, regress in response to castration, and subsequently regrow as castration-resistant (CR), PSA-producing variants that were utilized in the present studies. The CR variant of LuCaP35 has previously been termed LuCaP35V, but for consistency the CR variants of both LuCaP23 and LuCaP35 are now designated CR. All experiments involving animals were done in accordance with protocols approved by the University of Washington Institutional Animal Care Use Committee.

Castrate male CB-17 SCID mice (Charles River Laboratories) were implanted subcutaneously with 20 mm³ pieces of LuCaP23CR or LuCaP35CR tumors. When tumors reached 250 to 300 mm³ (length × (width²)/2) mice (n = 46 and n = 28 LuCaP23CR and LuCaP35CR tumor-bearing mice, respectively) were randomly assigned to vehicle control (5% benzyl alcohol, 95% safflower oil) and abiraterone treatment (0.5 mmol/kg/d in vehicle) daily for 21 days following enrollment (23). Unanticipated toxic or off target effects were not reported in the preclinical studies evaluating this dose (which is ~10-fold the oral dose in clinical studies, due to differences in absorption and bioavailability). Serum was collected by retro-orbital bleeding at interval time points for determination of PSA. Tumors from a subset of mice in each cohort were harvested at early time points of treatment (tumor size of ~500mm³, range 7–21 days). When the remaining tumors reached approximately 1,000 mm³ in size, animals were euthanized according to institutional protocol and xenografts harvested and flash frozen for determination of tissue androgens and extraction of total RNA. Serum PSA was measured using the Abbott AxSYM immunoassay system (Abbot Laboratories). Abiraterone was kindly provided by Cougar Biotechnology. Four mice bearing LuCaP35CR tumors survived for follow-up beyond day 40, whereas all mice bearing LuCaP23CR tumors reached the endpoint and were sacrificed by day 40 (except one at day 42).

Steroid measurements

Androgen levels were determined by mass spectrometry (MS) using methods we have recently described (24). This procedure resulted in a lower limit of quantitation of 1 pg per sample for testosterone and DHT, respectively. Intra-assay coefficients of variation generated using human serum for high-, mid-, and low-range samples were 3.5%, 3.1%, and 3.8% for testosterone and 6.3%, 4.3%, and 15.8% for DHT, respectively.

RNA isolation and quantitative real time-PCR

RNA was isolated and prepared for quantitative real time PCR (qRT-PCR) as previously described (5). cDNA was generated in a random-primed reverse transcription reaction, and qRT-PCR reactions were done in triplicate using an Applied Biosystems 7900 sequence detector with 5 ng of...
cDNA, 1 μmol/L of each primer pair and SYBR Green PCR master mix (Applied Biosystems). Primers (Supplementary Data 1) were designed using the Web-based primer design service Primer3 from the Whitehead Institute for Biomedical Research (http://www.steverozen.net/papers/rozen-and-skaletsky-2000-primer3.pdf), except for AKR1C2 and AKR1C3 (25) and RODH14, DHRS9 (NT-Salpa HSD), and 17BHSID10 (26) for which previously published primer sequences were used. Specificity of amplification was assessed based on melting point of the dissociation curve. In certain cases, 2.5 μL of amplified product (total reaction volume 10 μL) was also run on a 2% agarose gel to assess for product size (compared with positive control) and the presence of extraneous bands (using a Hamamatsu digital camera with acquisition software from LabWorks).

Statistical analysis

The effect of abiraterone on tumor growth was quantified using the following linear mixed effects model:

\[
\text{Volume}_{ij} = \mu + \beta_1 \text{Day}_{ij} + \beta_2 \text{Treatment}_{ij} + \beta_3 \text{Day}_{ij} \times \text{Treatment}_{ij} + b_i + \epsilon_{ij}
\]

where Volume_{ij} is log-transformed tumor volume measurement j for mouse i, Day_{ij} is day of measurement, Treatment_{ij} indicates treatment group (0 = none and 1 = Abiraterone), b_i is a mouse-specific independent and normally distributed random effect with mean 0 and variance \(\sigma^2_b\), and \(\epsilon_{ij}\) is an independent and normally distributed error term with mean 0 and variance \(\sigma^2_\epsilon\). Median tumor volume trajectories and 95% confidence intervals for each treatment group were compared using a generalized linear mixed model (GLMM) with a log link function and a normal distribution to assess for differences in tumor size over time.

Results

Abiraterone inhibits the growth of castration resistant prostate cancers

Prostate cancers progressing in the setting of castration maintain or reactivate the gene expression program regulated by the AR (27). In studies designed to identify mechanisms responsible for AR signaling in CRPC, we previously showed that tissue levels of T and DHT in LuCaP23CR and LuCaP35CR xenograft tumors grown in castrate mice are similar to tumor levels measured in isogenic variants passaged in noncastrate mice (5). To determine whether CYP17A1 activity contributes to tumor growth and the maintenance of tumor androgen levels, we treated cohorts of castrate mice bearing LuCaP23CR or LuCaP35CR xenografts with the CYP17A1 inhibitor, abiraterone.

Treatment with abiraterone led to a rapid decline in serum PSA over the first 10 days of treatment in mice bearing either LuCaP23CR or LuCaP35CR tumors (Fig. 1A and B). Abiraterone also had significant effects on tumor growth, with more rapid median growth per day in control versus Abi-T tumors (Fig. 2A and C; LuCaP23CR: 7.4% (95% CI: 6.2–8.0%) vs. 5% per day (95% CI: 3.0%–6.8%), \(P = 0.0001\); and LuCaP35CR: 4.8% (95% CI: 3.9–5.2%) vs. 2.5% per day (95% CI: 1.2%–3.7%), \(P < 0.0001\)). Accordingly, abiraterone treatment resulted in statistically significant improvements in PFS (defined as tumor size < 1,000 mm^3), and median survival (MS) in both xenografts (Fig. 1B and D). The median survival of LuCaP23 improved from 14 to 24 days [HR for survival 2.5 (95% CI: 1.6–11.2)], whereas the median survival of LuCaP35 improved from 17 to 39 days [HR 3.6 (95% CI: 2.3–34.6)]. Interestingly, serum PSA levels at tumor progression remained low in mice bearing LuCaP23CR tumors, although they began to rise in mice bearing LuCaP35CR tumors. These observations are consistent with a number of xenograft models showing that coordinate regulation of tumor growth and PSA expression is not necessarily universal (28).

Abiraterone treatment reduces tumor androgen levels in castration resistant prostate cancers

To determine the effect of abiraterone on tumor androgen levels, we measured levels of T and DHT in tumors resected at different time points during and after abiraterone treatment. At early time points during therapy (day 7 for LuCaP23 and days 7–21 for LuCaP35), abiraterone resulted in nearly complete suppression of T (Fig. 3A and B) and marked suppression of DHT (Fig. 3C and D) in both LuCaP23CR and LuCaP35CR tumors, respectively (\(P < 0.0001\) compared with controls for all comparisons). Levels of T (Fig. 3A) and DHT (Fig. 3C) remained suppressed at later time points in the majority of LuCaP23CR tumors. This included abiraterone-resistant (Abi-R) tumors that recurred within the 21 day treatment period (Abi-R), and tumors which recurred after therapy had been completed (Abi-T). In contrast, androgen levels in Abi-T LuCaP35 tumors (all of which recurred after day 21) showed a trend toward reconstitution, with a small but statistically significant increase in T (\(P = 0.032\), Fig. 3B) and a trend toward increase in DHT (\(P = 0.058\), Fig. 3D) compared with tumors resected earlier while on therapy (d7–21). Notably, tumor androgen levels in the abiraterone treatment arms were correlated with serum PSA levels in both LuCaP23CR and 35CR (\(r = 0.7168\), \(P < 0.0001\); and \(r = 0.9163\), \(P < 0.0001\); for Pearson correlations with tumor DHT, respectively), consistent with biologic activity associated with androgen levels in the recurrent tumors.
Abiraterone treatment alters the expression of full-length and splice-variant forms of the AR

Castration resistant prostate tumors frequently express elevated levels of AR<sup>FL</sup> and, as recently reported, increases in expression of AR splice variants that confer constitutive ligand-independent activity (2, 7–11, 12). To determine whether castration resistant tumors recurring after abiraterone treatment show further alterations in AR expression, we quantitated mRNAs encoding AR<sup>FL</sup>, and the AR<sup>del567es</sup> and AR<sup>V7</sup> variants recently identified in CRPC metastases.

Figure 1. Changes in serum PSA levels in LuCaP23CR and LuCaP35CR prostate cancer xenografts in response to treatment with abiraterone. Castrate male SCID mice were implanted subcutaneously with LuCaP23CR or LuCaP35CR tumors and randomly assigned to vehicle control or abiraterone treatment for 21 days. Serum PSA curves for control (blue) or Abi-T mice (red) are shown for individual mice bearing LuCaP23CR (A) and LuCaP35CR (B) xenografts. The segment of the y-axis denoted by the heavy bar in each graph is enlarged and presented in the adjacent panels on an expanded y-axis to more clearly show the decline in serum PSA over the first approximately 10 to 15 days after initiation of treatment.

Figure 2. Tumor growth and survival in LuCaP23CR and LuCaP35CR prostate cancer xenografts treated with abiraterone. Castrate male SCID mice were implanted subcutaneously with LuCaP23CR (A and B) or LuCaP35CR (C and D) tumors and randomly assigned to vehicle control or abiraterone treatment for 21 days. Median tumor volume trajectories with 95% confidence bands for control (blue) or Abi-T mice (red) bearing the LuCaP23CR (A) and LuCaP35CR (C) xenografts. Kaplan–Meier plots of PFS (defined as tumor size < 1,000 mm<sup>3</sup>) in control or Abi-T mice bearing the LuCaP23CR (B) and LuCaP35CR (D) xenografts. P values for curve comparisons generated using the Mantel–Haenszel log-rank test.
Abiraterone treatment alters the expression of AR-regulated genes and transcripts encoding steroidogenic enzymes

The ultimate objective of CYP17A1 inhibition by abiraterone is suppression of intratumoral androgens with concomitant inhibition of AR-mediated signaling. We therefore determined the impact of abiraterone on tumoral expression of androgen-regulated genes. We also evaluated expression of steroidogenic genes throughout the androgen biosynthetic pathway, as alteration in steroidogenic activity might mitigate the impact of abiraterone on intratumoral androgen levels.

Expression of androgen regulated genes

Consistent with decreases in tumor androgen levels associated with abiraterone treatment, both LuCaP23CR and LuCap35CR show significant decreases in expression of androgen-regulated genes (e.g., NKX3.1, FKBP5, TMPRSS2; Table 1). Interestingly, tissue PSA expression was not altered by abiraterone treatment in LuCaP23CR (Fig. 5A), remaining highly expressed despite the rapid and sustained suppression of serum PSA levels associated with abiraterone treatment (Fig. 1A). In contrast, treatment with abiraterone was clearly associated with inhibition of tissue PSA expression in LuCap35CR (Fig. 5B).

(Continued)
particularly at the early time points (d7-21) when tumors were under active treatment. Moreover, tissue and serum PSA levels were directly correlated in Abi-T LuCaP35CR tumors (Pearson correlation $r = 0.8226; P = 0.0006$) but not in LuCaP23CR. These observations suggest that androgen-mediated effects on serum PSA levels may reflect alterations in release/secretion of PSA with or without associated changes in tumoral PSA transcription.

Expression of genes required for androgen synthesis

Abi-T tumors showed increased expression of numerous genes throughout the androgen biosynthetic pathway (Fig. 6). Both LuCaP23CR and LuCaP35CR tumors responded to abiraterone treatment with increased expression of the target gene CYP17A1 (Fig. 5C, LuCaP23CR Abi-R 1.7-fold, $P = 0.0002$; Abi-T 2.1-fold, $P = 0.0001$; Fig. 5D, LuCaP35CR Abi-T 2.1-fold, $P = 0.0278$). The expression of AKR1C3 and HSD17B3, 2 key enzymes mediating conversion of adrenal androgen intermediates to T, was also increased in both LuCaP23CR and LuCaP35CR tumors compared with controls (Table 1). Of note, expression changes in Abi-R LuCaP23CR tumors recurring while on therapy were generally similar in direction and magnitude to changes observed in Abi-T tumors recurring after completion of abiraterone treatment (Abi-T), suggesting the effects of abiraterone were sustained beyond the immediate treatment interval.

Overall, genes required for androgen biosynthesis were expressed in both LuCaP23CR and LuCaP35CR tumors, and observed changes in expression are consistent with an upregulated capacity for androgen biosynthesis. As might be anticipated, the relative magnitude of steroidogenic gene induction in CRPC xenografts, before and after abiraterone treatment, is lower than that observed in clinical studies of CRPC tissues, in which CRPC metastases were compared with primary, untreated prostate tumors (5).
Expression of genes mediating prereceptor regulation of DHT levels

Tumor androgen levels reflect the sum total of activity in both androgen biosynthetic and androgen catabolic pathways. The back-conversion of 3α-diol to DHT, which can be mediated by enzymes with 3 alpha-hydroxysteroid reductase (3α-HSD) activity, has primarily been attributed to RL-HSD, although RODH4, RDH5, HSD17B10, and DHR9 can also mediate this reaction and are expressed to varying degrees in prostate tissue (29, 30). We therefore examined whether genes involved in prereceptor regulation of DHT levels via back conversion of 3-α-androstaneol (3-α-diol) to DHT were also altered by abiraterone treatment. Interestingly, changes in expression of oxidative genes mediating back conversion of 3α-diol to DHT in abiraterone-treated tumors were mixed, with increases in genes such as DHR9 in LuCaP23CR, and RDH5 in LuCaP35, but significant decreases in expression of RL-HSD in both LuCaP23CR and LuCaP35CR (Table 1).

The marked suppression of RL-HSD in response to ligand inhibition with abiraterone is consistent with a recent report showing suppression of RL-HSD levels in CWR22 Pc xenografts in response to castration (30), and may explain the relatively low levels of androgens measured in Abi-T tumors despite general induction of genes mediating androgen biosynthesis. Notably, tumor DHT levels were strongly correlated with RODH4 (r = 0.7348, P < 0.0001) and RL-HSD (r = 0.6431, P = 0.005) in LuCaP23CR, and with RODH4 (r = 0.6211, P = 0.0235), 17BHSD10 (r = 0.6332, P = 0.0202), DHR9 (r = 0.7815, P = 0.0016) and RL-HSD (r = 0.6329, P = 0.0202) in LuCaP35CR (Pearson product correlations). These data suggest that despite an overall decrease in expression of genes such as RL-HSD, androgen levels in abiraterone-treated tumors are, nevertheless, specifically associated with expression of genes inhibiting catabolism of DHT.

As tissue DHT levels in Abi-T LuCaP35CR tumors ranged from approximately 0.1 to >4 pg/mg (Fig. 4D), we also determined whether these differences in androgen reconstitutions were related to changes in the steroidogenic transcriptome. Notably, Abi-T LuCaP35CR tumors that recurred with high versus low tissue androgens (defined as tissue DHT above or below the median of ~0.5 pg/mg) showed 2.5-fold higher PSA (P = 0.007), 2.9-fold higher CYP17A1 (P = 0.033), markedly higher HNF4A (97-fold, P = 0.020, a CYP17A1 cofactor), and 8.2 and 5.4 higher levels of genes involved in preventing DHT catabolism (P = 0.011 and P = 0.005 for RL-HSD and DHR9, respectively), consistent with a critical role for genes involved in both androgen synthesis and prereceptor regulation of DHT catabolism in determining tissue androgen levels. (Statistically significant differences in expression of AR or AR variants were not observed, data not shown).

Discussion

Clinical studies with abiraterone have shown striking responses in men with CRPC, though not all patients respond and most patients eventually develop resistance. Although abiraterone is known to inhibit CYP17A1 in vitro, the effectiveness of abiraterone in suppressing intratumoral androgens has not been established, and it remains unclear which CRPC phenotypes and genotypes are susceptible to CYP17A1 inhibition and how tumors develop resistance. Using 2 distinct CRPC models, we show that treatment with abiraterone significantly inhibited tumor growth, serum PSA, and intratumoral androgen levels, supporting the hypothesis that abiraterone’s primary mechanism of action is through effects on tissue androgens. Furthermore, both CRPC models responded to CYP17A1 inhibition with mechanisms that maintain AR signaling. This included upregulated expression of AR variants and ligand-independent AR variants, as well as induction of steroidogenic genes (including the target gene, CYP17A1), several of which showed strong correlations with DHT levels in the recurrent tumors. Thus, in the setting of tumor progression on abiraterone, the rationale for focusing further therapeutic efforts on more potent AR antagonists and agents suppressing AR ligands remains strong.

Although specific mechanisms driving induction of alternative AR splicing have not been established, generation of AR splice variants following suppression of tumor androgens by abiraterone is consistent with the castration-mediated induction of AR splice variants observed in castration sensitive prostate cancer models (10, 12, 13). Interestingly, studies of testosterone replacement in this setting (either in vitro, or when given within days of castration in vivo) have been shown to inhibit castration-associated increases in AR variant expression (10, 12). However, we did not observe lower levels of full length or variant AR expression in those tumors with higher levels of androgens at recurrence (data not shown). These observations suggest that factors regulating the initial induction of AR splice variant expression could differ from those maintaining variant expression at later time points of recurrent growth. Moreover, these observations show that certain tumors may simultaneously engage or accrue multiple resistance pathways directed at preserving the AR axis.

The molecular alterations occurring in CRPC tumors following abiraterone treatment suggest tumor-specific methods of addressing resistance, either through optimizing steroidogenic blockade or by inhibiting AR signaling. Although substantially suppressed, androgen levels remained detectable in Abi-T tumors. Importantly, a 2- to 3-fold increase in expression of AR variant is known to render low androgen levels physiologically relevant in promoting AR driven growth (2), suggesting clinical treatment of Abi-R patients with more stringent ligand inhibition may be of benefit. More complete reduction of steroidogenesis might be achieved through enhancing local concentrations of CYP17A1 inhibitors, or targeting transcriptional activation of the enzyme. CYP17A1 is regulated by SF-1 and other cofactors (31), and its regulatory domains contain multiple cAMP responsive elements, providing several targets for modulation of enzyme expression, such as using phosphodiesterase inhibitors (32). Combining CYP17A1 blockade
### Table 1. Changes in AR and steroidogenic gene expression in Abi-T CRPC xenografts

<table>
<thead>
<tr>
<th>Time point of resection</th>
<th>Early (d7)</th>
<th>Abiraterone&lt;sup&gt;a&lt;/sup&gt; resistant (d11-21)</th>
<th>Abiraterone&lt;sup&gt;b&lt;/sup&gt; treated (d24-42)</th>
<th>Early (d7-21)</th>
<th>Abiraterone&lt;sup&gt;b&lt;/sup&gt; treated (d29-67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene and function</td>
<td>Fold&lt;sup&gt;d&lt;/sup&gt;</td>
<td>P&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Fold</td>
<td>P</td>
<td>Fold</td>
</tr>
<tr>
<td>AR</td>
<td>AR&lt;sub&gt;FL&lt;/sub&gt;</td>
<td>2.7</td>
<td>P &lt; 0.0001</td>
<td>2.3</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>AR&lt;sub&gt;del567&lt;/sub&gt;es</td>
<td>2.2</td>
<td>0.026</td>
<td>2.7</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>AR&lt;sup&gt;V7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>3.1</td>
<td>0.0003</td>
</tr>
<tr>
<td>AR-regulated genes</td>
<td>PSA</td>
<td>–1.2</td>
<td>0.026</td>
<td></td>
<td>–1.9</td>
</tr>
<tr>
<td>Cholesterol import</td>
<td>SCARB1</td>
<td>4.5</td>
<td>P &lt; 0.0001</td>
<td>7.1</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>and processing</td>
<td>LDLR</td>
<td>1.8</td>
<td>0.003</td>
<td>2.6</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>LIPE</td>
<td>1.2</td>
<td>0.031</td>
<td>1.6</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>SCAP</td>
<td>1.5</td>
<td>0.002</td>
<td>1.5</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>CYP11A1</td>
<td>1.8</td>
<td>0.003</td>
<td>2.4</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>STAR</td>
<td>–1.6</td>
<td>P &lt; 0.0001</td>
<td>–1.4</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>STARD3</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>STARD4</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>CYP17A1</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>CYP17B1</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>HNF1A</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>HNF4A</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>NR5A1 (SF-1)</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>CYB5A</td>
<td>1.5</td>
<td>0.005</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>POR</td>
<td>1.6</td>
<td>P &lt; 0.0001</td>
<td>2.1</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>DAX1</td>
<td>2.8</td>
<td>0.03</td>
<td>2.0</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>DUSP</td>
<td>1.7</td>
<td>0.026</td>
<td>-3.1</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Conversion of C-19</td>
<td>HSD3B1</td>
<td></td>
<td></td>
<td>1.7</td>
<td>0.063</td>
</tr>
<tr>
<td>steroids to T and DHT</td>
<td>HSD3B2</td>
<td></td>
<td></td>
<td>1.7</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>HSD17B3</td>
<td>1.3</td>
<td>0.006</td>
<td>1.4</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>AKR1C3</td>
<td>1.6</td>
<td>0.086</td>
<td>1.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>SDR5A1</td>
<td></td>
<td></td>
<td>–1.4</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>SDR5A2</td>
<td>1.9</td>
<td>0.028</td>
<td>3.5</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>SDR5A3</td>
<td>1.2</td>
<td>0.038</td>
<td>1.4</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

(Continued on the following page)
### Table 1. Changes in AR and steroidogenic gene expression in Abi-T CRPC xenografts (Cont'd)

<table>
<thead>
<tr>
<th>Time point of resection</th>
<th>Gene and function</th>
<th>LuCaP 23 CR</th>
<th>LuCaP 35 CR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early (d7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abiraterone\textsuperscript{a} resistant (d11-21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fold\textsuperscript{c}</td>
<td>P\textsuperscript{d}</td>
<td>Fold</td>
</tr>
<tr>
<td>CYP19A</td>
<td>1.7</td>
<td>0.009</td>
<td>2.5</td>
</tr>
<tr>
<td>AKR1C2</td>
<td>4.2</td>
<td>P &lt; 0.0001</td>
<td>3.9</td>
</tr>
<tr>
<td>HSD17B10</td>
<td>--2.9</td>
<td>0.000</td>
<td>--5.9</td>
</tr>
<tr>
<td>RODH 4</td>
<td>--4.8</td>
<td>P &lt; 0.0001</td>
<td>--26.2</td>
</tr>
<tr>
<td>RLHSD</td>
<td>--4.3</td>
<td>0.003</td>
<td>--26.2</td>
</tr>
<tr>
<td>&amp;HSD17B6</td>
<td>3.7</td>
<td>0.0001</td>
<td>7.5</td>
</tr>
<tr>
<td>RHDRS9 &amp;HSD17B13</td>
<td></td>
<td></td>
<td>--6.3</td>
</tr>
<tr>
<td></td>
<td>UGDH</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>UGT2B15</td>
<td>4.6</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>UGT2B17</td>
<td>2.6</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

|                         | Abiraterone\textsuperscript{b} treated (d24-42) |             |             |
|                         | Fold | P | Fold | P |
|                         | 2.5   | 0.037 | 3.3   | 0.0001 |

|                         | Early (d7-21) | Abiraterone\textsuperscript{b} treated (d29-67) |             |             |
|                         | Fold | P | Fold | P |
|                         | --1.8  | 0.024 | 2.8   | 0.0001 |

\textsuperscript{a}Abiraterone resistant tumors recurred (size 1,000 mm\textsuperscript{3}) and were resected while on abiraterone.

\textsuperscript{b}Abiraterone treated tumors recurred (size 1,000 mm\textsuperscript{3}) after the 21 day treatment period.

\textsuperscript{c}Fold change from difference in mean ΔC\textsubscript{T}'s (ΔΔC\textsubscript{T} method; fold = 2\textsuperscript{ΔΔC\textsubscript{T}}) between controls and Abi-T tumors in the indicated groups (early, resistant, or treated). Fold changes for genes which were not statistically significant are omitted.

\textsuperscript{d}P values from 2 sample t tests. The values of P ≤ 0.05 were considered significant. Genes with the values of P < 0.10 were considered trending toward significance if fold change was also 1.5 or more (shown in italics).
with inhibitors of other critical components of the pathway such as HSD3B1 or SRD5A1/2 could also offset adaptive upregulation of CYP17A1 (33).

Data regarding expression of C terminal truncated AR splice variants in CRPC continues to emerge, and will be a critical area of investigation as more potent ligand synthesis and AR inhibitors become utilized in the treatment of CRPC. AR splice variants may act by potentiating activity of ARFL and mediating constitutive AR transactivation (10, 13). Thus, increased expression of AR splice variants in Abi-T tumors may be an important biomarker of resistance and target for therapy. Incorporation of potent AR inhibitors, such as MDV3100, or agents targeting the AR N-terminal domain, such as EPI-001, could be utilized for tumors adapting to CYP17A1 inhibition via induction of ARFL and/or AR splice variants lacking the ligand-binding C-terminal domain (13, 34–36). Studies to delineate whether sequential or concurrent use of these agents with abiraterone can improve tumor growth inhibition and/or suppress adaptation in xenograft models will be important to provide rationale for human studies evaluating these agents in the treatment of clinical disease.

The response to abiraterone in our study is most likely due to suppression of de novo intratumoral steroidogenesis (due to the reported lack of CYP17A1 in rodent adrenal glands; refs. 37, 38), whereas in human studies the response to abiraterone may reflect inhibition of both adrenal and/or intratumoral CYP17A1 activity. Importantly, the proposed mechanism driving clinical activity of abiraterone in both scenarios is a decrease in intratumoral androgens (whether from suppression of adrenal steroidogenesis, intratumoral steroidogenesis, or both). Our observations confirm this expected mechanism of activity. Furthermore, increases in expression of ARFL and ligand-independent AR splice variants following abiraterone treatment are likely to be clinically relevant mechanisms of resistance regardless of whether tumoral CYP17A1 activity is present.

Another consideration for translation of our results to the clinical setting is that men with CRPC are likely to be treated with abiraterone plus prednisone (or dexamethasone) rather than abiraterone alone. In castrate men treated with abiraterone, glucocorticoids are primarily used to inhibit pituitary-mediated secretion of ACTH (induced by adrenal CYP17A1 inhibition) which can exacerbate side effects of mineralocorticoid excess, but may potentially inhibit tumor growth directly (39). ACTH may also drive clinically significant increases in production of androgenic precursors by the adrenal gland, as evidenced by PSA declines following the addition of dexamethasone in men who had progressed on abiraterone (15). Thus, the inclusion of prednisone in men with CRPC is likely to accentuate any decrease in tumor androgens caused by abiraterone, and may accentuate the types of tumoral responses observed in the Abi-T xenografts.

Our hypothesis that tumoral androgens in these xenograft studies are derived from de novo steroidogenesis is consistent with previous observation that a subset of CRPC
metastases have increased levels of transcripts for CYP17A1 and HSD3B1 (necessary for de novo synthesis) and the demonstration by Locke and colleagues that CRPC xenografts are capable of synthesizing DHT from acetate (5, 6, 40). However, alternative androgenic precursors (of adrenal or other origin) may also be present in the circulation as potential substrates. Unfortunately, serum samples adequate to assess circulating androgen levels were not available for analysis in this study. In addition, the duration of abiraterone treatment in our study was 21 days. It is possible that more prolonged treatment would have resulted in a more robust induction of the adaptive changes already observed. Finally, metastatic CRPC is characterized by significant heterogeneity, while our conclusions reflect an analysis of only 2 CRPC phenotypes, and we had small numbers in some of the treatment groups. These limitations can be addressed by evaluating diverse panels of CRPC xenografts over a more prolonged time course of therapy, and through studies of human tumor biopsies obtained at abiraterone resistance.

In conclusion, our finding that abiraterone suppresses intratumoral androgens and inhibits CRPC growth supports the hypothesis that tissue androgen levels are major contributors to AR signaling and mediators of CRPC progression. Though hypothesized, this is the first demonstration that the efficacy of abiraterone is related to its ability to suppress tumor androgen levels and complements previously published data from phase I studies regarding suppression of serum androgens (15, 41). Our results also identify potential mechanisms of adaptation to CYP17A1 blockade, including increased expression of AR, AR splice variants, and the steroidogenic transcriptome. Importantly,

Figure 6. The pathways of androgen biosynthesis. In the classical pathway (solid gray arrow), C21 precursors (pregnenolone and progesterone) are converted to the C19 adrenal androgens DHEA and androstenedione (AED) by the sequential hydroxylase and lyase activity of CYP17A1. DHEA and AED are converted to testosterone by a series of reactions involving the activity of HSD3B1 and 2, HSD17B3 and AKR1C3 enzymes. Testosterone is converted to the potent androgen DHT by the activity of SRD5A1 and 2. Oxidative 3α-HSD enzymes (including HSD17B6 (RL-HSD), HSD17B10, HSD17B13 (DHRS9), RODH4, and RDH5) can act to inhibit the prereceptor catabolism of DHT. An alternative pathway (short gray arrows) has also been proposed in which C21 precursors are first acted upon by SRD5A and the reductive activity of AKR1C2, followed by CYP17A1, HSD17B3, and subsequent oxidation to DHT. Adapted from ref. 42 with permission.
these adaptive mechanisms can potentially be targeted by using higher dose levels of abiraterone or combinations with potent AR antagonists currently in development. These data provide optimism that more effective suppression of AR signaling will continue to be an important means of effectively treating advanced prostate cancer.

Disclosure of Potential Conflicts of Interest

R.B. Montgomery received commercial research grant from Cougar Biotechnology. The other authors disclosed no potential conflicts of interest.

Acknowledgments

We thank Holly Nguyen, Andrew Morgan, Jared Lucas, and Roger Coleman for expert technical assistance; Roman Galati for statistical support; Cougar Biotechnology for providing abiraterone; and the Lucas Family for their generous support.

Grant Support

Prostate Cancer Foundation (Career Development Award to E.A. Mostaghel and Synergy Award to S. Balk, R.B. Montgomery, and P.S. Nelson); Damon Runyon Cancer Research Foundation (Damon Runyon-Gentest Clinical Investigator Award CI-40-08 to E.A. Mostaghel); NIH (Career Development Award K23 CA122820 to E.A. Mostaghel; Pacific Northwest Prostate Cancer SPORE P50 CA97186 (R.L. Vessella, P.S. Nelson); the Cancer Center Support Grant to Fred Hutchinson Cancer Research Center (P30 CA110774 paid to R.B. Montgomery)); and the Veterans Affairs Research Service (R.B. Pylmate).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 18, 2011; revised July 12, 2011; accepted July 14, 2011; published OnlineFirst August 1, 2011.

References


Resistance to CYP17A1 Inhibition with Abiraterone in Castration-Resistant Prostate Cancer: Induction of Steroidogenesis and Androgen Receptor Splice Variants


Clin Cancer Res 2011;17:5913-5925. Published OnlineFirst August 1, 2011.