Anticancer Activity of a Novel Selective CYP17A1 Inhibitor in Preclinical Models of Castrate-Resistant Prostate Cancer

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Abstract

VT-464 is a novel, nonsteroidal, small-molecule CYP17A1 inhibitor with 17,20-lyase selectivity. This study evaluates the anticancer activity of VT-464 compared with abiraterone (ABI) in castrate-resistant prostate cancer cell lines and xenograft models that are enzalutamide (ENZ)-responsive (C4-2) or ENZ-resistant (MR49C, MR49F). In vitro, androgen receptor (AR) transactivation was assessed by probasin luciferase reporter, whereas AR and AR-regulated genes and steroidogenic pathway enzymes were assessed by Western blot and qRT-PCR. The MR49F xenograft model was used to compare effects of oral VT-464 treatment to vehicle and abiraterone acetate (AA). Steroid concentrations were measured using LC-MS chromatography. VT-464 demonstrated a greater decrease in AR transactivation compared with ABI in C4-2 and both ENZ-resistant cell lines. At the gene and protein level, VT-464 suppressed the AR axis to a greater extent compared with ABI. Gene transcripts StAR, CYP17A1, HSD17B3, and SRD5A1 increased following treatment with ABI and to a greater extent with VT-464. In vivo, intratumoral androgen levels were significantly lower after VT-464 or AA treatment compared with vehicle, with the greatest decrease seen with VT-464. Similarly, tumor growth inhibition and PSA decrease trends were greater with VT-464 than with AA. Finally, an AR-antagonist effect of VT-464 independent of CYP17A1 inhibition was observed using luciferase reporter assays, and a direct interaction was confirmed using an AR ligand binding domain biolayer interferometry. These preclinical results suggest greater suppression of the AR axis with VT-464 than ABI that is likely due to both superior selective suppression of androgen synthesis and AR antagonism. Mol Cancer Ther; 14(1); 1–11. ©2014 AACR.

Introduction

Recent clinical trials have clearly established that castrate-resistant prostate cancer (CRPC) continues to remain sensitive to agents that inhibit the androgen receptor (AR) pathway (1, 2). Targeting androgen biosynthesis in CRPC dates back to the use of ketoconazole almost 25 years ago (3). More recently, abiraterone (ABI) acetate (AA) in combination with prednisone (6). The development of selective CYP17A1 lyase inhibitors has potential to obviate the need for concomitant prednisone administration in patients which is associated with its own toxicities.

CYP17A1 is the rate-limiting enzyme in the biosynthesis of androgens. CYP17A1 is a dual function enzyme with both 17-hydroxylase and 17,20-lyase functions sharing the same active site, which makes selective therapeutic inhibition of the lyase function more challenging. Inhibition of the hydroxylase function in patients results in an adrenocorticotropic hormone feedback-mediated mineralocorticoid excess which can be suppressed through prednisone administration (Supplementary Fig. S1). In addition to avoidance of side effects of prednisone, avoiding prednisone may have potential oncologic benefits in patients (7, 8).

VT-464 is a novel small-molecule CYP17A1 inhibitor with selectivity for the 17,20-lyase activity of this dual enzyme (9–11; Fig. 1A). The objective of this study was to evaluate the anticancer activity of VT-464 compared with abiraterone (ABI) in preclinical models of CRPC, particularly in the enzalutamide (ENZ)-resistant cell lines because preliminary clinical reports indicate that ABI is less effective in patients with a prior ENZ history (12, 13). We used both a CRPC cell line model (C4-2) and two previously reported (14) ENZ-resistant cell lines (MR49C, MR49F) to model advanced disease responses to this novel inhibitor and to ABI in vitro and in vivo.

Materials and Methods

Materials

Stock solutions of testosterone (T; Sigma), 4-androstene-3,17-dione (Sigma), 4-pregnen-17-ol-3,20-dione (Steraloids, Inc.).
tumor volume was >200 mm³. Two million MR49F cells with 0.5 mL Matrigel were inoculated in both flanks of mice for the pharmacodynamic study and in the left flank for the full study, similar to previous experiments (14). ENZ (10 mg/kg) was given by oral gavage on a 5-day on, 2-day off cycle starting the day of tumor inoculation and stopped at the time of treatment initiation. PSA levels were measured by tail vein sera samples weekly using the Cobas automated enzyme immunoassay (Montreal, PQ). Tumor measurements were made twice weekly using calipers (volume = length × width × height × 0.5326). Treatments began once the total tumor size exceeded 200 mm³. For the pharmacodynamic study, tumors were collected after 3 to 10 days (for each treatment: 2 mice after 3 days, 1 after 7 days, and 2 after 10 days) and fragments were either immediately frozen in liquid nitrogen, fixed in formalin, or preserved in RNAlater (Ambion). Treatments were cycled for 5 days on, 2 days off. All xenograft tumors were harvested approximately 3 hours after their last treatment dose. Mice were sacrificed when tumor volume exceeded 1,500 mm³ or loss of >20% body weight.

Figure 1.
A, molecular structure of AA and VT-464, a highly selective 4-(1,2,3-Triazole)–based P450C17a17,20-lyase inhibitor. B, immunoprecipitation demonstrating CYP17A1 protein in indicated LNCaP-based cell lines with densitometry quantification below.

In vitro models
C4-2 cells were provided as a gift from Dr. Leland Chung (1992; MD Anderson Cancer Center, Houston TX) and tested and authenticated by whole-genome and whole-transcriptome sequencing on Illumina Genome Analyzer Iix platform in July 2013. MR49C and MR49F cells were derived from LNCaP cells through serial xenograft passage in ENZ-treated mice as described previously (14). Cell lines were treated in RPMI 1640 in 10% charcoal-stripped serum (CSS; Hyclone), with 10 mol/L ENZ present in the media of MR49C and MR49F cells. For all 6-day experiments, media with corresponding treatments were changed at 72 hours.

In vivo models
All animal experimentation was conducted in accordance with institutional standards and the Canadian Council of Animal Care. Male athymic mice (Harlan Sprague-Dawley, Inc.) were castrated 1 to 2 weeks before tumor inoculation with ENZ 10 mg/kg being started the day before inoculation and continued until the total steroid analysis by LC-MS
Cell pellets and tissue homogenates were extracted 1:4 (v:v) with 60/40 hexane/ethyl acetate (hex/EtOAc) and media with EtOAc. Cell lines studies were performed in technical triplicates with biologic duplicate experiments and each tumor sample was measured in technical triplicates. Extracted steroids were dried (CentriVap) and reconstituted in 50 to 100 μL of 50 mmol/L hydroxyalnine, incubated for 1 hour at 65°C and the resulting oximes analyzed using a Waters Aquity UPLC Separations Module coupled to a Waters Quattro Premier XE Mass Spectrometer. Separations were carried out with a 2.1 × 100 mm bridged ethyl hybrid (BEH), 1.7 μm C18 columns, mobile phase water (A), and 0.1% formic acid in acetonitrile (B; gradient: 0.2 minute, 20%B; 8 minutes, 80%B; 9–10 minutes, 100%B; 10.2 minutes, 20%B; 12 minutes, run length). All data were collected in ESI+ by multireaction monitoring with instrument parameters optimized for the m/z’s and corresponding fragments of the oxime-steroids. Data processing was done with Quanlynx (Waters) and exported to Excel for addition normalization to weights and volumes as required. Deuterated T and DHT were used as internal standards (IS) and a curve of 6 calibration standards (0.01–10 ng/mL) used for quantification (R² > 0.98). Recoveries were greater than 80%, including extraction, conversion, and matrix effects. The extraction protocol was found to also be effective for VT-464 and ABI and they withstood the derivatization procedure essentially intact; however, some conversion of ABI to DHEA was observed (<0.01%) rendering samples very high in ABI unusable for that endpoint. Deuterated T and DHT were used as internal standard and a curve of 6 calibration standards (0.005–10 μmol/L, VT-464; 0.0015–3 μg/mL, ABI) used for quantification (R² > 0.98). Samples were diluted as needed with IS blank to be within calibration range.

PCR and Western blot analysis
Steroidogenic enzyme mRNA quantification in tumor samples was assayed by quantitative reverse transcription-PCR (qRT-PCR) with primers for STAR, HSD3B2, CYP17A1, HSD17B3, SRD5A1, and AKR1C3 (sequences in Supplementary Table S1). Reactions were conducted with 0.4 μL of RT-PCR cDNA, 0.8 μL each of forward and reverse primers (3 μmol/L), 2 μL nuclease-free water (Ambion), and 4 μL Roche SYBR Green qPCR MasterMix.
Triplicates of samples were run on the default settings of the ABIVii7 real-time PCR machine. For Western blot analysis, cell pellets or homogenized tumor tissue were processed in RIPA buffer (50 mmol/L Tris, pH 7.2, 1% NP-40, 0.1% deoxycholate, 0.1% SDS, 100 mmol/L NaCl and 1 × Roche complete protease inhibitor cocktail). Protein (30–50 μg) was loaded and run on gel electrophoresis. After washing thrice with washing buffer, membranes were incubated with Alexa Fluor 680 or 800 secondary antibodies (Invitrogen) for 1 hour. Detection of specific bands with their densitometric quantification was done using the ODYSSEY IR imaging system (Li-COR Biosciences). AR (sc-816/sc-7305), PSA (sc-7638/sc-69664), and CYP17A1 (sc-46084) antibodies were purchased from Santa Cruz Biotechnology and Vinculin (V9131) with an antibody from Sigma-Aldrich. For immunoprecipitation, total proteins were preclarified with protein-G sepharose (Invitrogen Life Technologies), immunoprecipitated with 2 μg anti-CYP17A1 before Western blotting as previously described (15). Western blots and qPCR analyses were performed in at least duplicate.

**AR transactivation assays**

C4-2, MR49C, or MR49F cells were plated on 6-well plates and transfected with 1.0 μg per well of ARR3-Luciferase plasmid using lipofectin (4.5 μL per well; Invitrogen Life Technologies, Inc.) overnight in serum-free media. Treatments in 10% CSS RPMI 1640 with VT-464, ABI, or DMSO control ± 0.1 mmol/L R1881 (PerkinElmer) were performed at indicated times. Luciferase activity (relative light units) was measured using a microplate luminometer (Tecan) in duplicate and normalized to the cell lysate protein concentration. All experiments were carried out in triplicate.

The AR agonist/antagonist activity of VT-464, ABI, and TAK-700 was assessed at Indigo Biosciences using Chinese hamster ovary (CHO) cells transfected with an expression vector that encoded a hybrid receptor comprised of full-length wild-type AR and an AR response element functionally linked to firefly luciferase. Reporter cell AR response was validated using 60-fluoro-testosterone as an agonist (EC_{50} = 60 pmol/L). For antagonist assays (n = 3), cells were mixed with 2 × the EC_{50} of 60-fluoro-testosterone and the CYP17 inhibitor. The percentage of antagonist inhibition was calculated as follows: 100 × [1 – (average relative light units (RLU) test compound/average RLU EC_{50} agonist)]. Dose–response nonlinear curve fits were generated using GraphPad Prism software, and IC_{50}s were calculated.

**Biolayer interferometry assay**

The direct reversible interaction between small molecules and the AR was quantified by biolayer interferometry (BLI) using OctetRED (Fortebio) as previously described (16). The ligand binding domain (LBD) of the biotinylated androgen receptor (bAR) was produced in situ with AviTag technology. The AviTag sequence (GLNDIFKEQEKWEH) followed by a six-residue glycine serine linker (GSGSGS) was incorporated at the N-terminus of the AR LBD (669-919). Escherichia coli BL21 containing both biotin ligase and AR LBD vectors were induced with 0.5 mmol/L isopropyl-β-D-thiogalactopyranoside in the presence of DHT and biotin at 16 °C overnight. The bacteria were then lysed by sonication, and the resulting lysate was purified by immobilized metal ion affinity chromatography with nickel_nitrilotriacetic acid (Ni_NTA) resin and cation-exchange chromatography (HiTrap SP). Purified bAR LBD (50 μg/mL) was bound to the streptavidin sensors over 5 minutes at room temperature. The sensor was kept in assay buffer [20 mmol/L N-2-hydroxyethylpiperezine-N0-2-ethanesulfonic acid, 150 mmol/L NaCl, 500 μmol/L tris(2-carboxyethyl)phosphine (TCEP), 500 mmol/L DHT, and 1% dimethylsulfoxide (DMSO)]. In all experiments, a known AR ligand was used as a control to confirm functionality of the bAR LBD (17).

**Statistical analysis**

Tumor growth velocity was calculated using linear regression of the log tumor volume over time. The Student t test was used to compare means. Means were plotted ± SEM. Significant differences [P ≤ 0.05 (**), P ≤ 0.01 (***)] were assessed using a Student t test. Kaplan–Meier curves compared OS and cancer-specific survival. Cancer-specific survival was defined by the time from treatment until animal sacrifice for tumor size exceeding endpoint.

**Results**

VT-464 suppresses the AR signaling pathway

The presence of CYP17A1 protein in our LNCaP-based cell lines was confirmed using immunoprecipitation with CYP17A1 antibody (Fig. 1B). To evaluate the activity VT-464 on AR signaling pathway, we first analyzed its effect on AR transactivation using an ARR3 luciferase reporter assay following treatment with 1, 5, and 10 μmol/L of ABI, VT-464, or control for six days in androgen-depleted media. In C4-2 cells, both ABI and VT-464 significantly decreased AR transactivation (Fig. 2A). At the 1 μmol/L and 5 μmol/L doses, this was significantly greater with VT-464 compared with ABI (P < 0.01). Notably, in the ENZ-resistant MR49C and MR49F cell lines, only VT-464 demonstrated decreases compared with control, whereas ABI was similar to controls or increased transactivation. With VT-464 treatment, a dose-dependent trend in decreasing transactivation was also observed in both MR49C and MR49F cells. Similar results were observed for all cell lines and doses after 72 hours of treatment (Supplementary Fig. S2A).

Because VT-464 induced a decrease in AR transactivation, we next assessed changes in AR-dependent genes, which were evaluated following six days in androgen-deprived conditions. The treatment-containing media were changed after 72 hours. In C4-2 cells, we found that both ABI and VT-464 stimulated an increase in AR mRNA transcripts, but a decrease in AR-dependent mRNA transcripts (e.g., PSA, NKG3.1) with greater decreases seen with VT-464 (Fig. 2B). In the ENZ-resistant MR49C cell line, ABI did not produce any decrease in AR-dependent transcript levels relative to control; VT-464 at equivalent doses lowered transcript levels compared with ABI. Similar to C4-2 cells, there was a VT-464-dependent decrease in AR-dependent genes compared with AR transcript levels, suggesting a feedback-induced increase in AR levels due to greater androgen suppression with VT-464. In MR49F cells, 10 μmol/L VT-464 induced a sharp decrease in AR and PSA mRNA (Fig. 2B) and PSA protein (Fig. 2C) possibly due to tumor cell apoptosis as evidenced by increased PARP cleavage (Supplementary Fig. S2B).

There were no differences in AR protein levels in C4-2 and MR49C cells following ABI or VT-464 treatment at the dose range tested, though AR levels did seem to increase more in response to VR-464 in MR49F cells as noted above (Fig. 2C). However, PSA protein levels were significantly lower after VT-464 treatment,
Figure 2.
Effect of VT-464 and ABI on the AR pathway. AR transactivation evaluated using a ARR3 luciferase plasmid (A). Transfected C4-2, MR49C, and MR49F cell lines were treated in androgen-depleted media for 6 days with 1, 5, or 10 μmol/L of treatment with ABI or VT-464 (VT). Results are normalized to total protein concentration. Representative results of at least three independent experiments ± SEM are presented. (Continued on the following page.)
with a dose-dependent decrease in the ENZ-resistant cell lines, compared with ABI-treated cells.

Changes in androgen synthesis pathway following CYP17A1 inhibition in vitro

Because VT-464 and ABI inhibit the AR signaling pathway by inhibiting CYP17 (9, 11), we evaluated mRNA transcript levels for selected steroidogenesis enzymes in all three cell lines following 6 days of treatment in androgen-depleted media. Both ABI and VT-464 treatments increased StAR, HSD3B2, HSD17B3, AKR1C3, and SRD5A1 enzyme transcript levels in a dose-dependent fashion (Fig. 3A); VT-464 was much more potent than ABI, presumably due to greater suppression of androgens. Similar trends were seen with upstream steroid and fatty acid pathway transcription factor SREBP-1 and HMGCR gene transcripts (Supplementary Fig. S3A–S3D). With the exception of outliers, the levels of CYP17A1 expression were higher in VT-464 than ABI-treated cells with no evident dose response observed (Fig. 3A).

Steroid level measurements were attempted for all cell lines but were only feasible in C4-2 cells due to detection limitations in MR49C and MR49F cells. Surprisingly, testosterone and DHT levels were increased in ABI-treated cells 6 days after treatment. This was consistent with the increase in PSA protein levels and AR and PSA mRNA transcripts observed in ABI-treated, but not VT-464–treated cells (Fig. 2B and C). Decreasing concentrations of progesterone and increasing concentrations of pregnenolone occurred with higher doses of ABI, consistent with prior reports of HSD3B2 inhibition with higher doses of ABI (ref. 18; Fig. 3B).

In C4-2 cells, the decreased AR transactivation with ABI compared with DSMO control, despite increased concentrations of the androgens DHT, T, and androsterone, as well as pregnenolone, suggesting that nonsteroid factors must be involved, such as drug AR antagonism. DHEA levels were very low in VT-464–treated cells, but could not be measured accurately in ABI-treated cells due to the low-level conversion of ABI to DHEA noted in the methods. Overall, these results indicated that in C4-2 cells, suppression of the AR pathway by ABI was not due to androgen concentration decreases. Further, for VT-464, the increase in steroid enzyme transcripts did not appear to be related to resistance as observed increases did not result in increased androgen biosynthesis or AR transactivation. In contrast, the increased androsterone and DHT that occurred in response to ABI in C4-2 cells does suggest that increased expression of AKR1C3 and SRD5A1 may be relevant to resistance through activation of alternate androgen synthetic pathways as previously reported (19, 20).

AR antagonism effect of VT-464

To investigate a possible AR antagonistic effect, we repeated transactivation assays with ABI and VT-464 in the presence of 0.1 nmol/L R1881. ENZ, as well as the CYP17 inhibitor TAK-700, was also tested as controls. Both ABI and VT-464 suppressed R1881 stimulation of AR transactivation (Fig. 4A). In C4-2 cells, the decrease in R1881-stimulated transactivation due to VT-464 was similar to that observed with ENZ, whereas no effect was seen with TAK-700. As expected, ENZ and TAK-700 again showed minimal changes in reporter activity in the ENZ resistance cell lines. Both ABI and VT-464 decreased R1881-induced AR transactivation in MR49F cells. Corroborating prior results, VT-464 demonstrated greater suppression of transactivation in the presence of R1881 than ABI in C4-2 and MR49C cells.

To further evaluate a direct antagonistic effect on the AR, VT-464 and ABI were tested in a wild-type AR luciferase assay transfected into CHO cells. AR antagonism was observed with both ABI and VT-464, but not with TAK-700 (Fig. 4B). Assessment of AR protein levels following VT-464 treatment at different time points and different doses indicated that AR degradation is not a major contributor to the AR antagonism observed (Fig. 4C).

A competitive binding assay using purified AR LBD and BLI demonstrated a clear dose-dependent effect, evidence of direct binding between VT-464 and the AR LBD (Fig. 4D).

VT-464 and AA decrease tumor growth and serum PSA levels in an in vivo model of ENZ resistance

Castrated mice inoculated with MR49F cells were randomized to oral gavage treatment with vehicle (0.5% methylcellulose) twice daily, AA 196 mg/kg twice daily, VT-464 75 mg/kg twice daily, and VT-464 150 mg/kg once daily. The AA oral gavage dose of 1 mmol/kg daily is higher than previously published doses of 0.5 mmol/kg/d (20), but was tolerated in prior unpublished experiments; the VT-464 dose of 0.375 mmol/kg daily (75 mg/kg twice daily or 150 mg/kg once daily) was used due to mouse weight loss at higher twice-daily doses. Efficacy of the VT-464 150 mg/kg once-daily arm was assessed preliminarily in 5 mice. AA and both VT-464 regimens demonstrated tumor growth inhibition (Fig. 5A). Mean tumor growth velocity was significantly lower in the VT-464 twice-daily arm compared with vehicle (P = 0.03); tumor growth velocity in AA arm did not differ statistically compared with vehicle (P = 0.06). Differences between AA and VT-464 were not significant (P = 0.64).

Serum PSA results were suppressed in all three treatment arms (Fig. 5B). After 3 weeks of treatment, the median PSA was lowest in the VT-464 twice-daily arm (Fig. 5B). The mean PSA velocity was significantly lower for AA compared with vehicle (P = 0.03), but not for VT-464 twice daily (P = 0.38). The waterfall plots after three weeks (Fig. 5B) show that there was a small subgroup of AA-treated mice with very low PSA that skewed the mean versus median values. Similarly, there were high PSA outliers in the VT-464 twice-daily treatment arm.

Results in the VT-464 150 mg/kg once-daily arm were generally similar to 75 mg/kg twice-daily regimen. Weight loss was similar between all treatment arms (Supplementary Fig. S5A). Two mice in the AA arm and one mouse in the VT-464 75 mg twice-daily arm were euthanized for weight loss >20% after 19, 33, and 39 days of treatment, respectively. One mouse in the VT-464 150 mg once-daily arm was found dead one day after starting treatment. Significant improvements in OS and cancerspecific survival were found only in the VT-464 75 mg/kg twice-daily arm compared with vehicle (P = 0.03 and P = 0.009, respectively; Fig. 5C).

Continued...
End-of-study intratumoral AR-dependent mRNA transcripts in VT-464 75 mg/kg twice-daily–treated mice were lower compared with AA (Supplementary Fig. S5B). End-of-study tumor samples also had steroidogenesis enzyme mRNA transcripts (Supplementary Fig. S5C), which were lower in both AA and VT-464 treatment arms compared with vehicle, with the lowest levels in the VT-464 arm.

End-of-study intratumoral AR-dependent mRNA transcripts levels (Supplementary Fig. S5B) were lower in VT-464 75 mg/kg twice-daily–treated mice compared with AA. ABI-treated mice...
had AR mRNA transcript levels slightly increased compared with vehicle-treated mice. Changes in AR-regulated genes PSA and NKX3.1 were also lower in the VT-464 compared with the AA arm, which were increased relative to vehicle. TMPRSS2 showed a different trend, with higher values in the VT-464-treated group compared with AA and vehicle. In contrast with the in vitro results above, select steroid synthetic enzyme mRNA transcripts (Supplementary Fig. S5C) were significantly lower intratumorally in
Pharmacodynamic study

To investigate pharmacodynamic effects, castrated mice implanted with MR49F xenografts in both flanks were treated orally with VT-464 at 100 mg/kg twice daily or AA at 196 mg/kg twice daily once tumors reached 200 mm³ in size as detailed in Materials and Methods. AR-dependent gene transcripts and AR and PSA protein level data collected from the tumors demonstrated significant heterogeneity of response (Fig. 6A and B), with a similar suppression of the AR pathway transcripts with both ABI and VT-464. Analysis of intratumoral drug levels indicated similar heterogeneity in intratumoral drug levels (Fig. 6C), though the intratumoral VT-464 concentrations were greater on average and somewhat less variable.

Figure 5.

*In vivo* evaluation of VT-464 and ABI. Castrated MR49F xenografts were randomized to treatment once tumor size exceeded 200 mm³. Treatments were vehicle (0.5% MC) twice daily (b.i.d), ABI 196 mg/kg twice daily, VT-464 75 mg/kg twice daily, and VT-464 150 mg/kg once daily (qd). Mean tumor size is plotted ± SEM (A, left). The waterfall plot details percentage change from baseline tumor size after 3 weeks of treatment for all mice in study (A, right). Waterfall plot of serum PSA after 3 weeks of treatment for all mice in study (B, left). Mean values ± SEM are plotted (B, right). Kaplan-Meier survival for all treatments: cancer-specific survival (C, left) and OS (C, right).
Analysis of intratumoral steroid levels demonstrated that testosterone and DHT levels were significantly lower following VT-464 or AA treatment compared with vehicle, with the greatest decreases seen with VT-464 (Fig. 6D). Upstream pregnenolone levels were significantly higher in tumors of AA-treated animals ($P = 0.03$).

Steroidogenic enzyme mRNA transcripts were assessed in the collected tumor samples. Here, in contrast with the prior in vivo findings, but similar to the in vitro findings, we found a trend toward increased steroid enzyme synthesis in AA- and VT-464-treated mice (Supplementary Fig. S6), with the greater
upregulation seen in the VT-464–treated mice, though androgen levels were lower in this group (Fig. 6D).

Discussion
This study demonstrated more potent inhibition of androgen synthesis by VT-464 in CRPC models compared with ABI particularly in the ENZ-resistant cells, MR49C and MR49F. Further, direct AR antagonism was demonstrated as a novel mechanism of action of VT-464. The use of LNCaP-based models mirrors the clinical situation wherein the T887A AR mutation renders AR antagonism limited by some heterogeneity. Nonetheless, this heterogeneity mirrors early clinical results of CYP17A1 inhibition in ENZ-resistant patients (12, 13).

In summary, the novel CYP17 inhibitor VT-464 demonstrated anticancer activity in preclinical models of CRPC and ENZ resistance, decreasing androgen levels significantly in castrate mice. The in vitro and in vivo results suggest greater suppression of the AR axis with VT-464 compared with ABI due to the selective suppression of androgen synthesis through CYP17 lyase inhibition, as well as AR antagonist effects.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Grant Support
P.J. Toren is supported by a Canadian Urological Association Scholarship Fund Award.

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Received June 19, 2014; revised October 17, 2014; accepted October 20, 2014; published OnlineFirst October 28, 2014.

References


Molecular Cancer Therapeutics

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Mol Cancer Ther Published OnlineFirst October 28, 2014.

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