Priority report

Interactions of abiraterone, eplerenone and prednisolone with wild-type and mutant androgen receptor: a rationale for increasing abiraterone exposure or combining with MDV3100

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ABSTRACT

Prostate cancer progression can be associated with androgen receptor (AR) mutations acquired following treatment with castration and/or an anti-androgen. Abiraterone, a rationally-designed inhibitor of CYP17A1 recently approved for the treatment of docetaxel-treated castration-resistant prostate cancer (CRPC), is often effective, but requires co-administration with glucocorticoids to curtail side effects. Here we hypothesized that progressive disease on abiraterone may occur secondary to glucocorticoid-induced activation of mutated AR. We found that prednisolone plasma levels in CRPC patients were sufficiently high to activate mutant AR. Mineralocorticoid receptor antagonists, such as spironoloactone and eplerenone that are used to treat side-effects related to mineralocorticoid excess, also bound to and activated signaling through both wild-type and mutant AR. Abiraterone inhibited in vitro proliferation and AR-regulated gene expression of AR-positive prostate cancer cells, which could be explained by AR antagonism in addition to inhibition of steroidogenesis. Interestingly, activation of mutant AR by eplerenone was inhibited by MDV3100, bicalutamide or greater concentrations of abiraterone. Therefore, an increase in abiraterone exposure above this threshold could reverse resistance secondary to activation of AR by residual ligands or co-administered drugs. Together, our findings provide a strong rationale for clinical evaluation of combined CYP17A1 inhibition and AR antagonism.

PRECIS

The administration of corticoid drugs intended to reduce the side effects of abiraterone in the treatment of CRPC can actually lead to disease progression via activation of wild-type or mutant androgen receptor.
INTRODUCTION

The small-molecule CYP17A1 inhibitor, abiraterone acetate (Zytiga, Jannsen), was recently approved for the treatment of men with castration-resistant prostate cancer (CRPC) progressing after docetaxel chemotherapy. Despite a significant survival advantage with 1000mg abiraterone daily and objective tumor responses in up to 60% of CRPC patients, progressive disease on treatment invariably develops (1, 2). MDV3100 is a novel anti-androgen (3, 4) that has also recently been reported to confer a survival advantage in CRPC patients progressing after docetaxel (5). As PSA often rises at progression on both these agents, we have hypothesized that resistance occurs secondary to reactivation of androgen receptor (AR) signaling. Inhibition of CYP17A1 results in significant suppression of androgens and estrogens but also of cortisol that is associated with a compensatory rise in adrenocorticotrophic hormone (2). Abiraterone acetate has therefore been developed in combination with exogenous glucocorticoids. However, up to 40% of patients on prednisone/prednisolone alone and 55% of patients on abiraterone acetate and prednisone/prednisolone develop a syndrome of secondary mineralocorticoid excess characterized by hypokalemia, hypertension and fluid overload that can be controlled by increasing the dose of prednisone or adding a mineralocorticoid receptor antagonist (MRA) such as eplerenone (1). Eplerenone is currently recommended in preference to spironolactone as previous studies demonstrated that eplerenone did not bind and activate wild-type (WT)-AR (2, 6). However, as eplerenone is not invariably available, spironolactone is also being used.

Point mutations of the AR, which appear to cluster in the ligand-binding domain, are rare in therapy-naïve patients but occur in 15-45% of castration-resistant disease and can increase AR affinity for a wide range of steroids (7, 8). Over 100 mutations have been described and many have been shown to give a functional advantage to maintain AR signaling. We hypothesized that progressive disease on abiraterone acetate could occur secondary to activation of mutated “promiscuous” AR by steroidal agents administered to patients to prevent or treat side-effects of mineralocorticoid excess.

MATERIALS AND METHODS

Materials
Fetal bovine serum (FBS) and charcoal-stripped, dextran-treated fetal bovine serum (CSS) were purchased from Gibco. Bicalutamide, dexamethasone, prednisone and dihydrotestosterone (DHT) (Sigma-Aldrich, UK), tritiated \[^{3}H\]-R1881 (Perkin-Elmer), R1881 (Steraloids, RI), eplerenone and spironolactone (Tocris-Bioscience, Bristol, UK) were obtained from commercial sources. Abiraterone and MDV3100 were synthesized using the publicly-available chemical structures and checked by mass-spectrometry. Drugs were dissolved in DMSO and then diluted to a maximum DMSO concentration of 0.2%. LNCaP, VCaP, PC-3, DU145 and COS-7 cells were obtained from ATCC-LGC Standards, grown according to ATCC recommendations, used less than 6 months from receipt and freeze-down and confirmed mycoplasma-free.

**Luciferase reporter assays**

We constructed a PSA-ARE3-luc luciferase reporter plasmid that was co-transfected with a human AR expression plasmid, F527-AR (wild-type (WT) or mutant as stated; mutations confirmed by sequencing (Beckman Coulter Genomics, UK)) into PC-3 cells. These were seeded in white opaque 384-well plates and grown in 10% CSS-supplemented phenol red-free RPMI 1640 for 30 hours. Cells were then treated with the indicated concentration of compound and R1881 for 16 hours. Luciferase activity was determined by adding ONE Glo (Promega, Southampton, UK) and measuring luminescence on a TopCount plate reader (Perkin-Elmer). Transfection efficiency and protein expression are shown in **Supplemental Figure 1**.

**Cell viability**

LNCaP and VCaP cells were seeded in 96-well plates and grown in CSS-supplemented phenol red-free or FBS-supplemented media for 7 days. Cells were treated with compound at 24 and 96 hours after plating and cell viability was determined on day 7 by adding CellTiter Glo (Promega) and measuring luminescence.

**Ligand-binding assay**

PC-3 cells transfected with WT or T877A mutant AR or LNCaP cells were seeded in 24-well plates and grown in CSS-supplemented phenol-red free media for 24 hours. To determine the kinetics of \[^{3}H\]-R1881 binding to the WT and T877A AR, cells were treated with 0.25-25nM \[^{3}H\]-R1881 for 2 hours, then washed, lysed and radioactivity was measured (1900CA analyzer, Perkin-Elmer). K\(_d\) and B\(_{max}\) were determined by nonlinear regression using Graphpad Prism™ software. When the concentration of \[^{3}H\]-R1881 required to almost saturate AR in both WT and
T877A AR mutant transfections was established (5nM), displacement of [3H]-R1881 by test compound was determined. The concentration at which 50% of [3H]-R1881 was displaced (EC50) was established using nonlinear regression (GraphPad Prism).

**Quantitative RT-PCR**

LNCaP and VCaP cells were seeded in 6-well plates and grown in CSS-supplemented phenol-red free media for 24 hours and then treated for 5 hours as indicated. Following RNA extraction and cDNA synthesis, quantitative-PCR was carried out on the Mx3000P QPCR System (Agilent) using the RT2 SYBR Green ROX™ qPCR Mastermix (SABiosciences). Every sample was run in duplicate and each reaction contained 50ng of cDNA in a total volume of 20µL. ΔCt for each gene was determined after normalization to actin and GAPDH and ΔΔCt was calculated relative to the designated reference sample. Gene expression values were set equal to 2–ΔΔCt (Applied Biosystems). Primers were purchased from SABiosciences.

**Measurement of plasma prednisolone**

Plasma was collected from CRPC patients after 48 days of continuous daily abiraterone acetate and prednisolone. All patients provided written, informed consent to blood withdrawal for research purposes and this study was approved by the Royal Marsden Hospital ethics review committees. Prednisolone was quantified by comparison to a calibration series ranging from 5 to 500ng/mL prepared in 50/50 methanol/water. A Waters Xevo mass spectrometer with Acquity uPLC system was used, fitted with a HSS T3, 1.8µm, 1.2x50mm column (Waters, UK). The column temperature was maintained at 60°C and the settings used were an electrospray source in positive ionisation mode; capillary voltage 4.0kV; source temperature, 150°C and desolvation temperature, 500°C.

**RESULTS**

**The selective mineralocorticoid-receptor antagonist, eplerenone, activates mutant AR**

We first co-transfected PC-3 AR-negative prostate cancer cells with PSA-ARE2-luciferase and either WT-AR or three mutations previously described in CRPC (T877A-AR, D879G-AR, W741C-AR). The T877A mutation has been identified in several studies in patients treated with flutamid (8, 9) and has been extensively studied as it is found in the LNCaP prostate cancer cell line (Supplemental Table 1). D879G and W741C mutations have been identified in
patients previously treated with bicalutamide (8, 9). We then compared activation of wild-type or mutant AR by synthetic androgen (R1881) to activation by the MRAs, eplerenone and spironolactone. In keeping with previous reports, spironolactone activates WT AR (7) and also T877A-AR, D879G-AR and W741C-AR only two-log less potently than R1881 does (Figure 1A, B and Supplemental Figure 2). Eplerenone does not activate WT-AR, D879G-AR or W741C-AR but importantly can activate T877A-AR with a dose proportional response and an EC50 of 5.2µM (95% CI: 2.89 to 9.37µM) (Figure 1A, B, Supplemental Figure 2). Pharmacokinetic studies with eplerenone report a Cmax of 1.72 ±0.28µg/ml (equivalent to 4.2µM ±0.7µM) and a half-life of 3 hours with 100mg eplerenone (6); doses of eplerenone between 50mg and 200mg are used to treat toxicities secondary to mineralocorticoid excess from abiraterone in CRPC patients (Supplemental Table 2). We proceeded to confirm that both spironolactone and eplerenone (1 and 10µM) increased proliferation of hormone-stripped LNCaP (T877A-AR) but only spironolactone increased the proliferation of VCaP (WT-AR) (Figure 1C). The increase in proliferation was inhibited by AR antagonism, suggesting this effect was secondary to binding to and activation of the AR (Figure 1C). Similarly, eplerenone significantly increased expression of the androgen-regulated and clinically important genes PSA and TMPRSS2 in LNCaP but not in VCaP (Figure 1D).

Exogenous glucocorticoids can activate mutant AR at clinically relevant doses observed in CRPC patients treated with abiraterone acetate

Prednisolone (UK) or its precursor prednisone (US) are commonly administered in combination with abiraterone acetate although two Phase II studies combined abiraterone acetate with dexamethasone (2, 10). Prednisone and dexamethasone do not activate WT-AR but activate T877A-AR with EC50s of 25.1µM (95% CI: 12.64 to 36.83µM) and 21.6µM (95% CI: 12.53 to 50.26µM) respectively (Figure 1A, B). Previous reports have demonstrated that other AR mutations such as T877A in combination with L701H are highly sensitive to glucocorticoids with activation by concentrations as low as 10nM (11). We therefore proceeded to measure plasma levels of prednisolone in 15 CRPC patients on continuous daily treatment with 1000mg abiraterone acetate and 10mg prednisolone. Prednisolone levels were <4nM in two patients but >30nM in the other 13 patients. The median concentration was 153nM (range: <4 to 305nM) (Figure 2, Supplemental Table 2).

Abiraterone binds and inhibits wild-type and mutant AR
Following the observation of activation of T877A-AR by eplerenone, we proceeded to evaluate the effect of abiraterone on wild-type and mutant AR (T877A, D879G, R629Q, W741C, M749L). We did not observe an increase in reporter-luciferase activity with doses of abiraterone up to 25µM with WT-AR or any mutation tested (Supplemental Figure 3) but observed dose-proportional inhibition of stimulated WT and mutant AR activity (Figure 3A) with significant inhibition observed at doses ≤10µM. Inhibition was however not as potent as for same concentrations of MDV3100. We then proceeded to confirm our findings by comparing inhibition of AR activation using abiraterone or MDV3100 in a different model system (COS-7 cells co-transfected with AR and a GRE2-TATA-Luc reporter gene and activated by 10nM DHT for 24 hours). Similarly we observed dose-proportional inhibition of WT-AR, T877A-AR, G142V-AR, P533S-AR, T575A-AR and H874Y-AR by abiraterone (Figure 3B). Higher concentrations of abiraterone were required for inhibition of R629Q-AR in this system than was observed in PC-3 cells transfected with an ARE3-luciferase assay (Figure 3A). We also confirmed significant inhibition of proliferation of the AR-positive prostate cancer cell lines LNCaP and VCaP with doses of abiraterone ≥5 µM (Figure 3C). No inhibitory effect was observed with the AR-negative prostate cancer cell lines, PC-3 and DU145 (Supplemental Figure 4). We proceeded to confirm down-regulation by quantitative-PCR of PSA and TMPRSS2 in LNCaP cells treated with abiraterone (Figure 3D).

**Binding of abiraterone or eplerenone to the AR is confirmed by competitive displacement of [3H]-R1881**

To confirm that AR antagonism by abiraterone and agonism by eplerenone (both previously undescribed) occurred secondary to binding to the AR ligand-binding domain, we used a competitive radiolabelled assay to demonstrate displacement of R1881 from PC-3 cells transfected with either WT-AR or T877A-AR. The EC\textsubscript{50} of eplerenone for WT-AR was six-fold higher than T877A-AR (EC\textsubscript{50}: 2.4 µM (95% CI 2.0-2.9 µM) (Figure 4A,B). In keeping with the inhibitory activity of abiraterone observed in our reporter-luciferase studies, abiraterone displaced ligand from both WT-AR (EC\textsubscript{50}: 13.4µM, 95% CI: 10.3-17.4µM) and T877A (EC\textsubscript{50}: 7.9µM, 95% CI: 6.7-9.3µM) (Figure 4A,B). We also confirmed displacement of radiolabelled R1881 from LNCaP with abiraterone (EC\textsubscript{50}: 2.6µM, 95% CI: 1.0–6.8µM) and eplerenone (EC\textsubscript{50}: 4.3µM, 2.4-7.8µM) (Supplemental Figure 5).
Mutant AR activation by eplerenone can be inhibited by abiraterone or bicalutamide but most effectively by MDV3100

We observed dose-proportional growth inhibition with abiraterone of LNCaP cells stimulated by eplerenone and of LNCaP and VCaP cells stimulated by spironolactone (Figure 1C). Similar levels of inhibition were observed with bicalutamide, with more profound inhibition by MDV3100 (Figure 1C). Abiraterone, MDV3100 and bicalutamide achieved similar levels of inhibition of up-regulation of PSA by eplerenone but MDV3100 inhibited induction of TMPRSS2 expression more significantly than bicalutamide or abiraterone (Figure 3D). Similarly, MDV3100 showed more significant inhibition of spironolactone-stimulated PSA and TMPRSS2 expressions than abiraterone or bicalutamide (Supplemental Figure 6). Also, abiraterone (5µM) significantly inhibited activation of T877A-AR (in transfected PC-3) by 1µM eplerenone but not by 10µM eplerenone; stimulation by 10µM eplerenone was significantly inhibited by both bicalutamide and MDV3100 (Figure 4C).

Increased hormone levels reduce AR inhibition by MDV3100

Recent studies have demonstrated that intra-tumoral testosterone levels rise in patients treated with MDV3100 (12). We found that ≥1µM and ≥10µM MDV3100 significantly inhibited WT AR luciferase activity stimulated by 0.1nM R1881 or 1nM DHT respectively but ≥50 µM MDV3100 was required to significantly inhibit AR stimulated by 1nM R1881 (Figure 4D) or 10nM DHT (Supplemental Figure 7).

DISCUSSION

Abiraterone was developed as a specific CYP17A1 inhibitor (13). Previous studies have failed to identify binding of abiraterone to the AR (14). However, in this study we utilized both reporter-luciferase and competitive radiolabelled assays to demonstrate that abiraterone binds and inhibits WT-AR. Another study published whilst our manuscript was under review reported supporting evidence that abiraterone binds the AR and produces a dose-dependent decrease in AR levels (15). This study failed to identify the EC50 with wild-type or mutant AR but predicted it as over ≥3µM. We also tested eight AR mutations selected from a screen of 42 mutations for causing a differential response to various hormones. We included mutations in the amino terminal (G142V, P533S), DNA-binding (T575A) and ligand-binding (W741C, M749L, T877A, D879G and H874Y) domains and the hinge region (R629Q) (Supplemental
Table 1). As previously described, bicalutamide activated W741C (4, 16) but no agonistic activity was observed with any mutation and abiraterone. Similarly MDV3100 potently inhibited WT-AR and mutant AR. However, these mutations were mostly identified in patients progressing on bicalutamide or flutamide and different, new mutations may develop in patients progressing on abiraterone or MDV3100.

Abiraterone is an active treatment for CRPC due to CYP17A1 inhibition and significant suppression of hormones (2). However, we observed up to 32% AR inhibition with 1µM abiraterone, with significantly greater inhibition at 5 and 10µM. Pharmacokinetic studies have reported maximum plasma levels after a single 1000mg dose of abiraterone acetate in fasting patients of 1.2 to 5µM, confirming AR antagonism could occur at clinically achievable doses (Supplemental Table 2) (2, 17). Higher doses of abiraterone up to at least 2000mg daily are safely tolerated (2) and greater activity could be observed with increased drug exposure despite complete CYP17A1 inhibition at lower doses. This could be achieved by administration with food (2, 17). Moreover, several studies of abiraterone have now reported preclinical in vitro and in vivo anti-tumor activity and inhibition of AR nuclear localization and AR-regulated transcription that was attributed entirely to inhibition of steroidogenesis (18, 19) but could in fact be partly explained by AR antagonism. Similarly, in vitro inhibition of LNCaP and VCaP cells in our study could also be explained by abiraterone’s effect on steroidogenesis.

Significant activation of both WT and mutant AR is observed with spironolactone that should be avoided in all CRPC patients. We also demonstrate activation of T877A-AR by eplerenone that was developed as a novel, non-AR binding MRA (6). This could underlie clinical resistance in a proportion of patients. Similarly, exogenous glucocorticoids that are currently administered in combination with abiraterone reach levels in patients that have been previously shown to activate mutant AR (11). Activation of “promiscuous” AR by co-administered drugs or residual hormones (as we reported recently (20)) could be inhibited by increasing the dose of abiraterone or possibly more effectively, combining with a potent antiandrogen such as MDV3100. Due to toxicity the dose of MDV3100 selected for Phase III development was 160mg daily that achieves median plasma concentrations up to approximately 35µM (3, 4). AR inhibition at these concentrations could be overcome by a rise in hormones that would be prevented by combination with abiraterone acetate. Overall these observations provide a strong rationale for clinical evaluation of combined CYP17A1 inhibition and AR antagonism.
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FIGURE LEGENDS

Figure 1. Eplerenone activates T877A-AR and spironolactone activates both T877A-AR and wild-type (WT)-AR. Sigmoidal dose response curves show activation of (A) WT-AR by R1881 and spironolactone and (B) T877A-AR by R1881, spironolactone, eplerenone, prednisolone, dexamethasone. Fold change from the DMSO control was plotted and EC50s calculated using non-linear regression (GraphPad). EC50 and 95% confidence intervals are given. C, LNCaP and VCaP prostate cancer cells in CSS were treated with eplerenone or spironolactone alone or in combination with 0.1, 1 or 5µM abiraterone, 10µM bicalutamide or 10µM MDV3100 or for 7 days and then analyzed for cell viability. Fold change from the DMSO control was then calculated and plotted. Significance is shown for stimulation by eplerenone or spironolactone compared to DMSO control and for inhibition by bicalutamide, MDV3100 or abiraterone when compared to stimulated levels. D, LNCaP and VCaP cells were treated with 0.1nM R1881 or 0.1 - 10µM eplerenone for 5 hours. RNA was extracted and cDNA synthesized for analysis by quantitative PCR to determine relative levels of PSA and TMPRSS2 mRNA expression. Significance compared to DMSO controls is shown. Data shown for all experiments are the mean (error bars, standard error of the mean, SEM) of 3 independent experiments of 16 replicates (A, B) or in duplicate (C, D). *, P<0.05; **, P<0.01; ***, P<0.001, one-way ANOVA with Bonferroni correction.
Figure 2. Plasma concentrations (nmol/L) of prednisolone in 15 CRPC patients treated with abiraterone acetate measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS). The median concentration of 152nM (SD 100nM) is marked by the solid line. The 10nM limit above which activation of T877A-L701H-AR has been previously reported to occur is shown by the dashed line.

Figure 3. Inhibition of wild-type and mutant stimulated AR activity by abiraterone, bicalutamide and MDV3100. A, PC-3 cells were co-transfected with ARE3-luciferase and WT or mutant AR (T877A, D879G, W741C, M749L, R629Q). Cells were treated with 0.1 - 25µM abiraterone, 10µM bicalutamide or 10µM MDV3100 in CSS medium containing 0.1nM R1881 for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was calculated and then percentage change relative to the R1881-stimulated DMSO control was determined. Data shown are representative of 3 independent experiments and represent mean and SEM of 8 replicates. B, COS-7 cells were co-transfected with GRE2-TATA-Luc and the WT or mutant human expression plasmid pSVARo (T877A, G142V, P533S, T575A, H874Y, R629Q). Cells were treated with 0.1 - 5µM abiraterone or MDV3100 in CSS medium containing 10nM DHT for 24 hours. The luciferase activities were assayed in duplicate and normalized for the amounts of expressed AR determined immunologically by dot blot analysis and normalized for protein concentration. The change in normalized luciferase activity relative to cells incubated without any compound for each AR variant was determined. Data shown represent two or three independent experiments performed in quadruplicate. C, Dose-proportional inhibition of proliferation of LNCaP and VCaP cells by abiraterone, MDV3100 and bicalutamide. LNCaP and VCaP prostate cancer cells in CSS with 0.1nM R1881 were treated with 0.1, 1 or 5µM abiraterone, 10µM bicalutamide or 10µM MDV3100 for 7 days and then analyzed for cell viability. Fold change from the DMSO control was then calculated and plotted. Data shown are the mean (error bars, standard error of the mean, SEM) of 3 independent experiments in quadruplicate. D, LNCaP cells were treated with 0.1nM R1881 or 10µM eplerenone in combination with DMSO, 10µM bicalutamide, 10µM MDV3100 or 5µM abiraterone for 5 hours. RNA was extracted and cDNA synthesized for analysis by quantitative PCR to determine relative levels of PSA and TMPRSS2 mRNA expression. Data shown are the mean and SEM of 3 independent experiments in duplicate. Significance is shown for *, P<0.05; **, P<0.01; ***, P< 0.001; ****, P<0.0001 relative to DMSO control (one-way ANOVA with Bonferroni correction).
Figure 4. Displacement of [3H] R1881 by eplerenone and abiraterone in PC-3 cells transfected with WT or T877A mutant AR. PC-3 cells were transfected with WT AR (A) or T877A mutant AR (B) and then treated with CSS media containing 5nM of [3H] R1881 in combination with cold R1881, DHT, eplerenone, abiraterone or bicalutamide at the concentrations shown for 2 hours. Abiraterone was insoluble in cell media at concentrations greater than 25µM. Cell-associated radioactivity was measured and the data analyzed by nonlinear regression to determine the EC50 for each test compound (GraphPad Prism). Data shown are the mean and SEM of three independent experiments in triplicate for percentage (%) [3H]-R1881 bound versus log10 of concentration (µM) of cold competitor. EC50 and 95% confidence intervals are given. C, Inhibition of eplerenone-stimulated AR activation by bicalutamide, MDV3100 and abiraterone. PC-3 cells were co-transfected with ARE3-luciferase and T877A mutant AR. Cells were treated with DMSO (control) or eplerenone in combination with DMSO, 10µM bicalutamide, 10µM MDV3100 or 5µM abiraterone for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was calculated. Data shown are from three independent experiments and represent mean and SEM of 13 replicates. D, Increased hormone levels reduce AR inhibition by MDV3100. PC-3 cells were co-transfected with ARE3-luciferase and WT-AR. Cells were treated with R1881 in combination with DMSO or MDV3100 at the concentrations indicated for 16 hours and then analyzed for luciferase activity. Fold change from the DMSO control was calculated. Data shown are from three independent experiments and represent mean and SEM of 24 replicates. ***, P< 0.01 relative to R1881 or DHT control with DMSO (one-way ANOVA with Bonferroni correction).
Figure 1

A

Log10 concentration (µM)

Fold change from DMSO control

R1881 EC50 15pM (95% CI 11 - 22pM)
Spironolactone EC50 61nM (95% CI 44 - 85nM)
Eplerenone
Dexamethasone
Prednisone

B

Log10 concentration (µM)

Fold change from DMSO control

R1881 EC50 35pM (95% CI 23 - 54pM)
Spironolactone EC50 6.9nM (95% CI 4.7 - 10nM)
Eplerenone EC50 5.2µM (95% CI 2.9 - 9.4µM)
Dexamethasone EC50 21.6µM (95% CI 12.5 - 50.3µM)
Prednisone EC50 25.1µM (95% CI 12.6 - 36.6µM)

C

Fold change from DMSO control (no R1881)

0.1µM R1881
0.1µM spironolactone alone
+ Abiraterone (µM)
+ 10µM bicalutamide
+ 10µM MDV3100
0.1µM Eplerenone alone
1µM Eplerenone alone
10µM Eplerenone alone
+ Abiraterone (µM)
+ 10µM bicalutamide
+ 10µM MDV3100

VCaP

LNCaP

D

Relative mRNA expression

Eplerenone
R1881

PSA
TMPRSS2
PSA
TMPRSS2

LNCaP
VCaP
Figure 2
Figure 3

A

% change in luminescence compared to DHT alone

WT AR  T877A  D879G  W741C  M748L  R629Q

B

% change in luminescence compared to DHT alone

WT AR  T877A  G142V  P533S  T575A  H874Y  R629Q

C

Vehicle alone  Abiraterone (µM)  Bicalutamide (µM)  MDV3100 (µM)

LNCaP  VCaP

D

Vehicle alone  + 5µM abiraterone  + 10µM bicalutamide  + 10µM MDV3100

Relative PSA mRNA expression  Relative TMPRSS2 mRNA expression

PSA  TMPRSS2

R1881 alone  + 5µM abiraterone  + 10µM bicalutamide  + 10µM MDV3100  Eplerenone alone  + 5µM abiraterone  + 10µM Bicalutamide  + 10µM MDV3100
Interactions of abiraterone, eplerenone and prednisolone with wild-type and mutant androgen receptor: a rationale for increasing abiraterone exposure or combining with MDV3100

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