Synergistic Targeting of PI3K/AKT Pathway and Androgen Receptor Axis Significantly Delays Castration-Resistant Prostate Cancer Progression In Vivo

Christian Thomas¹, Francois Lamoureux¹, Claire Crafter², Barry R. Davies², Eliana Beraldi¹, Ladan Fazli¹, Soojin Kim¹, Daksh Thaper¹, Martin E. Gleave¹, and Amina Zoubeidi¹

Abstract

The progression to castration-resistant prostate cancer (CRPC) correlates with gain-of-function of the androgen receptor (AR) and activation of AKT. However, as single agents, AR or AKT inhibitors result in a reciprocal feedback loop. Therefore, we hypothesized that combination of an AKT inhibitor with an antiandrogen might result in a more profound, long-lasting remission of CRPC. Here, we report that the AKT inhibitor AZD5363 potently inhibits proliferation and induces apoptosis in prostate cancer cell lines expressing the AR and has anticancer activity in vivo in androgen-sensitive and castration-resistant phases of the LNCaP xenograft model. However, we found that the effect of castration-resistant tumor growth inhibition and prostate-specific antigen (PSA) stabilization is transient and resistance occurs with increasing PSA after approximately 30 days of treatment. Mechanistically, we found that single agent AZD5363 induces increase of AR binding to androgen response element, AR transcriptional activity, and AR-dependent genes such as PSA and NKX3.1 expression. These effects were overcome by the combination of AZD5363 with the antiandrogen bicalutamide, resulting in synergistic inhibition of cell proliferation and induction of apoptosis in vitro, and prolongation of tumor growth inhibition and PSA stabilization in CRPC in vivo. This study provides a preclinical proof-of-concept that combination of an AKT inhibitor with antiandrogen results in prolonged disease stabilization in a model of CRPC. Mol Cancer Ther, 12(11): 2342–55. ©2013 AACR.

Introduction

Although early detection and treatment of localized prostate cancer has improved, many men still die of metastatic disease. Almost 80% of patients initially respond to androgen-deprivation therapy (ADT), most patients progress to castration-resistant prostate cancer (CRPC) and metastatic disease (1–6). CRPC is a complex process by which cells survive and proliferate in low-circulating androgen. This in part involves the reactivation of the androgen receptor (AR) axis (7), by pro-survival genes and alternative mitogenic growth factor pathways (8, 9) including the phosphoinositide 3-kinase (PI3K)/AKT pathway that offers substantial therapeutic potential in prostate cancer (10, 11). This pathway is upregulated in prostate cancer in high-risk disease and CRPC (10, 12, 13). The existence of cross-talk between the PI3K/AKT pathway and the AR axis has been proposed, however the underlying molecular mechanisms are still unknown (14–18).

Although targeting the PI3K/AKT pathway by small-molecule inhibitors has been reported to be a promising approach to delay prostate cancer progression in the clinic (9, 17, 18), the upregulation of the AR pathway seems to counter its effect in prostate cancer (15, 16, 18). For instance, Carver and colleagues showed that combined inhibition of PI3K/AKT and AR signaling using the PI3K/mTOR inhibitor BEZ235 and the antiandrogen MDV3100 resulted in an enhanced antitumoral activity in the hormone-sensitive LNCaP mouse model (18). However, it is not known whether the combined blockade of AR and PI3K/AKT pathway will effect tumor progression in the castration-resistant state. The aim of the current study is to evaluate the antitumoral activity of the novel AKT inhibitor AZD5363 at different stages of prostate cancer both in vitro and in vivo. Here, we show that AZD5363 potently affects cell growth and survival of AR-positive prostate cancer cells in vitro. The underlying mechanisms are based on induction of caspase-3–dependent apoptosis. Interestingly, PI3K/AKT pathway inhibition by AZD5363 leads to an upregulation of AR-dependent proteins. AZD5363 inhibits...
cancer progression in the hormone-sensitive as well as in the CRPC LNCaP xenograft model. Most importantly, combination treatment of AZD5363 plus bicalutamide circumvents AZD5363-induced AR upregulation and results in long-lasting inhibition of CRPC progression. The data support further investigation of the therapeutic potential of AZD5363 in human prostate cancer, particularly in combination with antiandrogens.

Materials and Methods

Prostate cancer cell lines and reagents

The human prostate cancer cell lines LNCaP and C4-2 used in this study were kindly provided by Dr. Leland W. K. 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 2009. Western blotting and/or real-time PCR was conducted for AR and prostate-specific antigen (PSA) each time when LNCaP or C4-2 cells were resurrected. After resurrection, both cell lines were passaged for less than 3 months. LNCaP and C4-2 cells were maintained in RPMI-1640 medium (Invitrogen) supplemented with 5% charcoal-stripped serum (CSS) or 5% FBS and cultured without antibiotics at 37°C in 5% CO₂ atmosphere.

Treatment of prostate cancer cells with AZD5363 and bicalutamide

The AKT inhibitor AZD5363 and the nonsteroidal antiandrogen bicalutamide were kindly provided from AstaZeneca and used, respectively, for in vitro and in vivo. AZD5363 is a new ATP-competitive inhibitor of AKT and, as a synthetic small molecule, orally available (19, 20). For the in vitro studies, AZD5363 was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at 10 mM stock solutions and stored at −20°C. For the in vivo studies, AZD5363 was dissolved in H₂O and 10% DMSO and 25% KLEPTOSE (Roquette Pharma) at 100 mg/mL and stored at room temperature for 1 week. For in vivo use, bicalutamide was dissolved in DMSO and stored for 4 weeks at room temperature. In vivo, bicalutamide was diluted with H₂O and used at 10 mg/kg body weight.

Cell proliferation and apoptosis assays

Cell growth was assessed using crystal violet assay, as described previously (19). Cells were plated in 96-well plates and treated with AZD5363 in FBS supplemented media. Crystal violet staining was carried out for dose- and time-dependent treatment. Cell proliferation after treatment was calculated as the percentage of the absorbance in untreated cells.

Chromatin immunoprecipitation (ChIP) assay was conducted as we previously described (19). The AKT gene as we previously described (21). The promoter region of the AR gene was assessed by flow cytometric analysis. Cells were plated in 10 cm² dishes using FBS-supplemented media and treated as described earlier. Three days after treatment, cells were trypsinized and fixed in 70% ethanol overnight at 4°C, then incubated with 1 µg/mL RNase A (Sigma) for 30 minutes at 37°C before stained with 5 µg/mL propidium iodide (Sigma) for 30 minutes at room temperature. The stained cells were analyzed for relative DNA content on a dual laser flow cytometer (Beckman Coulter Epics Elite; Beckman, Inc.). Each assay was conducted in triplicate.

Western blot analysis

Cell population was harvested in a dose-dependent manner after AZD5363 treatment at 5 µmol/L in radioimmunoprecipitation assay buffer (RIPA) buffer (50 mmol/L Tris, pH 7.2, 1% NP-40, 0.1% deoxycholate, 0.1% SDS, 10 mmol/L NaCl, Roche complete protease inhibitor cocktail) and Western blots were conducted as described previously (19) using antibodies as described in Supplementary Table S1.

Quantitative reverse transcription PCR

Total RNA was extracted from cultured cells after 48 hours of treatment using TRIzol reagent (Invitrogen Life Technologies, Inc.). Real-time monitoring of PCR amplification of cDNA was conducted as previously described (21) on ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) with SYBR PCR Master Mix (Applied Biosystems). Each assay was conducted in triplicate.

Luciferase assay

LNCaP and C4-2 cells were plated on 12-well plates and transfected with PSA-luciferase-plasmid (−6,100 to +12) using lipofectin (Invitrogen Life Technologies, Inc.). The total amount of 1.0 µg/well PSA-plasmid DNA was used. Sixteen hours before start of treatment media was changed into CSS ± 0.1 mmol/L R1881 (PerkinElmer). Luciferase activity were measured using the Dual-Luciferase Reporter Assay System (Promega) using a microplate luminometer (EG&G Berthold). Luciferase activity was normalized to the protein concentration of the cells. All experiments were carried out in triplicate wells and repeated two times.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assay was conducted as previously described (22). Briefly, LNCaP cells were cross-linked with paraformaldehyde and digested with micrococcal nuclease to achieve a DNA smear of 200 to 1,000 bp. ChIP assay was conducted using SimpleChIP Enzymatic Chromatin IP Kit (Agarose Beads) according to the manufacturer’s protocol (Cell Signaling Technology) on the AR gene as we previously described (21). The results are representative of at least three independent experiments.
Figure 1. AZD5363 inhibits prostate cancer tumor growth and induces cell-cycle arrest and apoptosis in vitro. A, effect of AZD5363 on the expression of distal AKT pathway biomarkers and biomarkers of AKT pathway output in LNCaP and C4-2 cells. B, LNCaP and C4-2 cells were treated with 10 nmol/L to 10 µmol/L AZD5363 for 24 to 72 hours. Cell viability was determined by crystal violet assay (left). Columns are means of triplicate analysis ± SE. Dose-dependent inhibition of cell growth by AZD5363 compared with perifosine, each dosed from 10 nmol/L to 10 µmol/L (right). Seventy-two hours after treatment, cell viability was assessed by crystal violet assay. Data points are means of triplicate analysis ± SE. (Continued on the following page.)
Assessment of synergy on cell viability

A combination index (CI) for synergy was determined by comparing the growth inhibition of bicalutamide (range, 10.0–50.0 μmol/L) or AZD5363 (range, 100.0–500.0 nmol/L) with that of the combination of both drugs (constant ratio 1:100) based on the median effect principle. This commonly used method was first described by Chou and Talalay (23). LNCaP and C4-2 cells were plated in 96-well plates containing CSS-supplemented media + 0.1 nmol/L R1881 and treated with both drugs as mentioned before. Cell viability inhibition was determined 72 hours after treatment by crystal violet assay and each experiment was repeated in triplicate.

Drug interactions were quantified with CalcuSyn software (BIOSOFT). CI was calculated for the effective doses ED₉₀, ED₇₅, and ED₉₅. A CI < 1 indicates synergy, a CI > 1 indicates antagonistic interactions, and a CI value more than 1 indicates additive effects. Moreover, dose reduction indices (DRI) was calculated for bicalutamide.

Animal treatment

In vivo, we used two different animal models to mimic hormone-sensitive and CRPC progression. For both models, male athymic nude mice (Harlan Sprague Dawley, Inc.) were injected subcutaneously with 2 × 10⁶ LNCaP and C4-2 cells were plated in 96-well plates containing CSS-supplemented media + 0.1 nmol/L R1881 and treated with both drugs as mentioned before. Cell viability inhibition was determined 72 hours after treatment by crystal violet assay and each experiment was repeated in triplicate.

Drug interactions were quantified with CalcuSyn software (BIOSOFT). CI was calculated for the effective doses ED₉₀, ED₇₅, and ED₉₅. A CI < 1 indicates synergy, a CI > 1 indicates antagonistic interactions, and a CI value more than 1 indicates additive effects. Moreover, dose reduction indices (DRI) was calculated for bicalutamide.

Results

AZD5363 targets the PI3K/AKT pathway and inhibits cell proliferation

The effects of the AKT inhibitor AZD5363 (Fig. 1A) on distal pathway biomarkers in vitro were investigated by Western blot analysis. As shown in Fig. 1B, AZD5363 treatment induced AKT⁴⁷³ and AKT₃₀⁸ phosphorylation but inhibited phosphorylation of the distal AKT-pathway biomarkers including PRAS40, elf4E, 4E-BP1, mTOR, and P70 S6 kinase in a time-dependent manner in both LNCaP and C4-2 cells. As a direct downstream effector to AKT, phosphorylation of S6 kinase was completely abrogated by AZD5363 6-hour posttreatment. This effect was
accompanied with a decrease in cell proliferation in a dose and time-dependent manner on both LNCaP and C4-2 cells (Fig. 1C). The effect of AZD5363 was evaluated in an extended panel of prostate cell lines. Prostate cancer cell lines expressing AR were more consistently sensitive to AZD5363 than lines with loss of PTEN. For example, AR+/PTEN wild-type LAPC-4 cells were much more sensitive to AZD5363 than AR-/PTEN null PC3 cells (Supplementary Table S2).

AZD5363 treatment induces apoptosis in prostate cancer cells in vitro

We next evaluated the effects of AZD5363 treatment on cell apoptosis. As shown in Fig. 1D, AZD5363 treatment significantly increased the fraction of cells undergoing cell death (sub-G0-G1) in a dose-dependent manner compared with control in both C4-2 and LNCaP cell lines (3.8%, 6.3%, 23.1%, 34.5%, and 61.6% in LNCaP and 2.2%, 3.6%, 13.3%, 25.0%, and 51.8% in C4-2 cells at 0 nmol/L, 100 nmol/L, 500 nmol/L, 1 μmol/L, and 5 μmol/L AZD5363, respectively). Moreover, we found that AZD5363 induces caspase-3 activity in time-dependent manner in LNCaP and C4-2 cells compared with control (Fig. 1D) and PARP cleavage (Fig. 1E). These results suggest that AZD5363 inhibit cell proliferation and induces apoptosis in a caspase-3-dependent manner.

AZD5363 treatment delays tumor progression to CRPC in vivo

Because AZD5363 induces apoptosis and inhibits cell proliferation in LNCaP cells, we set out to determine whether AZD5363 has efficacy in vivo. We first evaluated the effect of AZD5363 in hormone-sensitive model. Basically, 1 day after castration, mice were randomized and treated with 100 mg/kg AZD5363 or vehicle orally twice a day. We found that AZD5363 significantly suppressed tumor growth (\( P < 0.01 \)) compared with control (Fig 2A, left) without any significant impact on average body weight (Supplementary Fig. S2). Individual analysis of tumor volume (Fig 2A, right) showed that after 10 weeks of treatment, 8 of 10 mice in the control group developed a tumor volume more than 500 mm² compared with 2 of 12 mice in the AZD5363 group. AZD5363 treatment significantly (\( P < 0.05 \)) affected serum PSA levels (Fig. 2B, left). The serum PSA velocity increased by 23.0 ng/mL per week in the control group, which was significantly higher (\( P < 0.05 \)) compared with 5.4 ng/mL per week in the AZD5363-treated mice (Fig. 2B, right). AZD5363 treatment significantly improved TPFS (\( P < 0.001 \)) and delayed median time to tumor progression from 18 days to 59 days (Fig. 2C). Two mice in the control group required sacrifice after 10 weeks (tumor volume > 10% of body weight), whereas no animals required intervention in the AZD5363-treated group. To further confirm pharmacodynamic activity in vivo, we conducted Western blot analysis and IHC analysis on random tumors, as shown in Fig. 3B, PSA protein levels were higher in the control group compared with the AZD5363-treated group. Although total AKT protein was expressed uniformly in both groups, the phosphorylated isoforms AKT\(^{T 473} \) and AKT\(^{T 308} \) (Fig. 2D, right) were upregulated in the AZD5363-treated animals, which is in concordance with our in vitro studies (Fig. 1A), suggesting a feedback loop. However, the distal pathway biomarkers output, pS6 and cyclin D1, were downregulated by AZD5363 compared with control and was further confirmed by IHC analysis showing that in xenografts, pS6 was inhibited in the AZD5363-treated animals. Moreover, Ki67 as a biomarker for cell proliferation was lower in the AZD5363 arm compared with control and cell death was more in the AZD5363-treated tumors as shown by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining (Fig. 2D, left).

AZD5363 treatment delays CRPC progression in vivo

We next evaluated the effect of AZD5363 on CRPC progression in vivo. Basically, mice were castrated when PSA reached 50 to 75 ng/mL and were randomized into treatment groups when serum PSA relapsed to precastration levels. In Fig. 3A, the average tumor volume was significantly higher (\( P < 0.01 \)) in the control group compared with the AZD5363-treated group and the tumor growth rate more than 5 weeks was significantly faster (\( P < 0.01 \)) in the control group compared with AZD5363-treated mice (206.8 mm²/week and 33.4 mm²/week, respectively). Moreover, AZD5363 treatment significantly (Fig. 3B; \( P < 0.05 \)) reduced serum PSA levels and serum PSA velocity compared with control (82.8 and 16.4 ng/mL/wk, for vehicle and AZD5363-treated groups, respectively). TPFS significantly (\( P = 0.0005 \)) improved by AZD5363 treatment compared with control. The median time to tumor progression was 14.5 days in the control group and not reached in the treatment group within the first 30 days of treatment (Fig. 3C). In concordance with the hormone-sensitive model, AZD5363 induces a decrease of cell proliferation as measured by Ki67, increased cell death as measured by TUNEL staining, and a decreased phosphorylation of S6 compared with controls (Fig. 3D). However, after approximately 30 days, the mean tumor size and serum PSA concentration in animals treated with AZD5363 exceeded the size of the controls at the start of the experiment, indicating tumor progression and onset of resistance (Fig. 3E). These data show that treatment benefit with AZD5363 in this model is short lived and resistance/relapse occurs with rising PSA levels, suggesting that AR is still active.

AZD5363 treatment activates the AR pathway

To explore the mechanism of treatment relapse, we evaluated the effect of AZD5363 on the AR signaling pathway. To our surprise, we found that inhibition PI3K/AKT pathway using AZD5363 increased AR expression in a time-dependent manner at both mRNA and protein levels (Fig. 4A). Similar results were observed with PI3K/mTOR inhibitor, rapamycin (Supplementary
Figure 2. AZD5363 inhibits prostate cancer tumor growth in the hormone-sensitive LNCaP model. A total of 2 × 10^6 LNCaP cells were injected subcutaneously in male athymic nude mice. Animals were castrated when PSA values exceeded 50 ng/mL and treatment started with 100 mg/kg AZD5363 or vehicle orally twice a day 5 times per week. A, the mean tumor volume of all mice (left) per group ± SE and for each mouse individually per group (right). **, significance at least P ≤ 0.01 for all time points between day 7 and 70. B, mean serum PSA levels of all mice per group ± SE (left). *, significance at least P ≤ 0.01 for all time points between day 7 and 70. AZD5363 treatment downregulates PSA protein expression in tumors. Vinculin was used as a loading control. The serum PSA velocity (ng/mL/week) was estimated by the slope more than 10 weeks. Columns represent average tumor growth rate (mm^3/week) ± SE, respectively. **, P ≤ 0.01. C, Kaplan–Meier survival analysis comparing AZD5363 versus control for TPFS. D, immunohistochemistry on xenograft tumors for pS6, Ki67, and TUNEL (left). Total proteins were extracted from xenografts treated with AZD5363 or control after 10 weeks or when tumor volume exceeded ≥ 10% (right). Samples were taken 4 hours after last AZD5363 treatment and Western blot analysis was conducted using pAKT/AKT, pS6/S6, and vinculin was used as a loading control.
Fig. S3) showing increased both AR and PSA expressions at protein levels. Consistent with these data, others have found that inhibition PI3K/mTOR pathway using BEZ235 results in upregulation of AR expression (18). We found that AZD5363 and rapamycin also induced extracellular signal–regulated kinase (ERK) phosphorylation (Supplementary Fig. S3). This pathway may play a critical role in the cross-talk between the PI3K/AKT pathway and the AR axis. We next evaluated whether AZD5363 induces activation of AR signaling pathway. We found that AZD5363 increased AR transcriptional activity in a dose-dependent manner (Fig. 4B) associated with enhanced target genes expression at mRNA level (PSA and NKX3.1; Fig. 4C). To further corroborate our finding on the effect of AKT inhibition pathway on AR, we specifically downregulated AKT using siRNA and found that AKT knockdown induced increase of PSA expression at protein level (Supplementary Fig. S3C) supporting that targeting AKT activates the AR pathway. To further understand how targeting AKT activates AR, we conducted ChIP and found that AZD5363 increased AR binding to the ARE I and ARE III sequences in PSA promoter (28) compared with control (Fig. 4D), which was accompanied by an increase in AR transcriptional activity. Azd5363 inhibits tumor growth in the castration-resistant phase of the LNCaP xenograft model. A total of 2 × 10^6 LNCaP cells were injected subcutaneously in male athymic nude mice. Treatment was started with 100mg/kg AZD5363 or vehicle orally twice a day 5 times per week when serum PSA relapsed to precastration levels. A, mean tumor volume (left) of all mice per group ± SE and tumor growth rate (mm³/week; right), B, mean serum PSA levels of all mice (left) per group ± SE and serum PSA velocity (ng/mL/week; right). **, P ≤ 0.01. C, Kaplan–Meier survival analysis comparing AZD5363 versus control for TPFS. D, immunohistochemistry on xenograft tumors. Samples were taken 4 hours after last AZD5363 treatment. E, mean tumor volume of all mice per group ± SE. After a tumor stabilization within the first 30 days of treatment, AZD5363-treated tumor start to progress again.
AZD5363 (1 µmol/L)  
Biclutamide (5 µmol/L)

C4-2LNCaP
PARP
AR
PSA
Akt
p-Akt
Vinculin

Figure 4. AZD5363 induces AR signaling, which is abrogated by bicalutamide. LNCaP cells were treated with 1 µmol/L AZD5363 in a time-dependent manner. mRNA and proteins levels were analyzed for AR and PSA. B, effect of AZD5363 treatment at indicated concentration on genomic activity of AR. LNCaP cells were transfected with PSA luciferase and treated with AZD5363 in media containing CSS ± 0.1 nmol/L R1881. Luciferase activity was measured over time using a luminometer. C, LNCaP cells were treated with 1 µmol/L AZD5363 in a time-dependent manner. mRNA levels were analyzed by quantitative reverse transcription PCR (RT-PCR) for AR target genes (PSA, FKBP5.2, and NKX3.1). D, LNCaP cells were treated with or without 1 µmol/L AZD5363 ± 0.1 nmol/L DHT. ChIP assays were conducted on nuclear extracts from LNCaP cells using 2 µg of the AR antibody and 20 µL of ProteinG agarose. The RT-PCR was conducted using immunoprecipitated DNAs, soluble chromatin, and specific primer pairs for the ARE I and ARE III promotor. E and F, LNCaP and C4-2 cells were treated with 1 µmol/L AZD5363 and/or 5 µmol/L bicalutamide for 48 hours. mRNA levels were analyzed by quantitative RT-PCR for AR and AR target genes (E) and protein levels were analyzed by Western blot analysis for the indicated antibodies (F). Bic, bicalutamide.
in AR nuclear translocation (Supplementary Fig. S4), AR transcriptional activity, and expression of AR target genes as we showed in Fig. 4B and C. Interestingly, the effect of AZD5363 on AR activation was abrogated with the AR antagonist bicalutamide. Hence, bicalutamide abrogates AZD5363-induced AR target genes expression at mRNA and protein levels (Fig. 4E and F), which was associated with increased PARP cleavage (Fig. 4F). These results suggest that combined inhibition of the AR signaling pathway using bicalutamide enhances the activity of AZD5363.

**Bicalutamide synergizes with AZD5363 to inhibit cell viability and induction of apoptosis in AR-positive prostate cancer cell lines in vitro**

Because AZD5363 in combination with bicalutamide (Fig. 5A) induced PARP cleavage, we tested whether this combination has an effect on cell proliferation. As shown...
AZD5363 + bicalutamide strongly reduced AR expression. These data collectively suggest that delayed tumor progression in AZD5363 + bicalutamide–treated mice results from both reduced proliferation and increased apoptosis.

Discussion

The PI3K/AKT pathway plays a major role in prostate cancer progression (10). It is upregulated in 30% to 60% of prostate cancer, especially at high Gleason score and in CRPC (12, 13). Involved in numerous cellular processes including cell growth and survival, pharmacologic targeting of the PI3K/AKT pathway might be a promising approach to affect prostate cancer growth. Several small-molecule inhibitors targeting different proteins of the PI3K/AKT pathway have shown potent anticancer activity in prostate cancer in vitro and in vivo including, GSK690693, perifosine and isoflavones (GCP; refs. 9, 17, 29–31). For instance, GSK690693 showed significant inhibition of tumor growth by 50% compared with control in LNCaP xenograft model (9). The isoflavone genistein significantly reduced tumor volume and incidence in the same tumor model (17). Furthermore, antitumoral effects of AKT inhibitors in subcutaneous PC3 xenograft mice have been reported in several studies (29, 32, 33).

So far, perifosine and celecoxib with AKT-inhibitory properties have been tested clinically in patients with advanced prostate cancer. Perifosine is a nonselective AKT inhibitor whereas celecoxib is a COX-2 inhibitor with additional AKT pharmacology; both compounds do not show any measured activity in clinical trials. For instance, perifosine induced PSA stabilization only in 1 of 5 patients with metastatic CRPC after 12 weeks of treatment and no patients showed any PSA decrease (34). These results were consistent with data from patients with biochemically recurrent prostate cancer treated with perifosine (35). The COX-2 inhibitor celecoxib used in combination with hormone therapy failed to enhance the activity of castration in patients with hormone-sensitive prostate cancer (36).

These conflicting data between preclinical and clinical studies could be the consequence of poor selectivity and suboptimal AKT pharmacology of the earlier compounds, or the use of inappropriate in vivo models that do not reflect the clinical course of prostate cancer. Antitumor response should not only be measured by tumor volume but also by serum PSA. This biomarker is of major importance to predict clinical benefit in patients with progressive prostate cancer (37). Moreover, ADT should be implemented in an in vivo model as it is standard treatment in metastasized prostate cancer (2, 38). Previous preclinical studies with other AKT inhibitors have not met these criteria. For example, in the previously mentioned LNCaP xenograft models serum PSA levels were not evaluated and the PC3 xenograft model does not express the AR, hence does
not represent the majority of CRPC in humans. Carver and colleagues (18) were able to show that combining castration with the PI3K/mTOR inhibitor BEZ235 induces regression of tumors in mice, whereas average tumor volume in mice treated with vehicle or BEZ235 monotherapy is increased (18).

Figure 6. Combination treatment of AZD5363 plus bicalutamide significantly delays CRPC tumor progression and prolongs cancer-specific survival compared with each monotherapy in vivo. On the basis of the CRPC model described in Fig. 4, animals were treated with control, AZD5363 (100 mg/kg twice a day), bicalutamide (10 mg/kg daily), or the combination of both. A, the mean tumor volume of all mice per group ± SE. P < 0.01. B, individual tumor progression in the different treatment groups. Change of tumor volume in percentage (%) of each mouse between day 1 and day of sacrifice was calculated. Each column displays individual change. C, mean serum PSA levels of all mice per group ± SE. P < 0.01. D, Kaplan–Meier survival analysis comparing AZD5363 versus control for cancer-specific survival. E, immunohistochemistry on xenograft tumors. Bic, bicalutamide.
In the current study, both in vivo experiments are based on the subcutaneous LNCaP xenograft model, which mimics the clinical course of prostate cancer in humans (37–39). Using the novel small molecule AZD5363, an ATP-competitive inhibitor of AKT, we are able to delay in vitro and in vivo prostate cancer progression in hormone-sensitive as well as in castration-resistant state. The antitumor activity of AZD5363 is based on cell-cycle inhibition and induction of apoptosis.

This study shows that AKT inhibition induces a delay of tumor progression to CRPC in xenograft model. AZD5363 significantly slows down tumor growth rate and serum PSA velocity by 83.9% and 80.0%, respectively (Fig. 3A and B). However, this effect is not long-lasting in the castration-resistant state. After 4- to 5-week treatment with AZD5363, xenograft tumors and serum PSA start to progress, showing that AZD5363 as a single agent induces a tumor stabilization for some time rather than sustaining tumor regression. It has already been described in the literature that resistance to AKT inhibitors might be induced by reactivation of the AR axis (13–15, 17). We show that specific pharmacologic inhibition of AKT signaling using AZD5363 induces a feedback regulation of the AR pathway (Fig. 4A–D). In concordance with other studies (40, 41), we suggest that the bidirectional cross-talk between AR and AKT is a rationale for simultaneous molecular targeting of both pathways. We identified phospho-ERK (pERK) as a potential link between the PI3K/AKT pathway and the AR axis (Supplementary Fig. S3A) and this is due to a feed-forward loop involving activation of EGF receptor and insulin-like growth factor receptor (Supplementary Fig. S3B) after AKT inhibition similar to the one reported by Chandarlapaty and colleagues in breast cancer (41). Because AKT has been reported to phosphorylate the AR on Ser-210 and Ser-790 and inhibits its transactivation (42), targeting AKT phosphorylation will then result in inhibition of AR phosphorylation on Ser-210 and Ser-790 and induce AR transactivation, which supports our finding that targeting AKT phosphorylation with AZD5363 will abrogate AR phosphorylation and increase the expression of AR-dependent genes such as PSA and NKx3.1, as seen in Fig. 4.

To optimize the clinical response to AKT pathway inhibition, we evaluated the therapeutic effect of AZD5363 combined with AR pathway inhibition using bicalutamide. AZD5363 induced upregulation of the AR-axis, which was abrogated by simultaneous treatment with antiandrogens. In the current study, we combined AZD5363 plus bicalutamide. In vitro, AZD5363 plus bicalutamide synergized on inhibition of cell viability (Fig. 5A). Moreover, induction of apoptosis was enhanced by combination treatment compared with each monotherapy treatment (Fig. 5C and D). Notably, the addition of bicalutamide prevented AZD5363 induced upregulation of the AR-axis (Fig. 4E and F).

Previous data have shown that combination treatment with antiandrogens and mTOR inhibitors enhance anticaner activity (15, 17, 43). However, these studies are not mimicking CRPC setting. We show here for the first time, the addition of bicalutamide significantly enhances the anticaner effects of AZD5363 in a CRPC xenograft model. Even after 70 days of treatment, tumor volumes in the combination arm do not exceed the values at the beginning (Fig. 6A). Also, serum PSA levels are significantly lower in the combination arm compared with each monotreatment (Fig. 6C). Most important, no animal in the combination arm had to be sacrificed within 70 days of treatment, whereas 50% of mice in the AZD5363 arm and 60% in the bicalutamide arm were sacrificed because of high-tumor burden (Fig. 6D). This study impressively shows that the synergistic effects of AZD5363 and bicalutamide lead to long-lasting tumor remission in the castration-resistant state. Moreover, it provides further evidence that targeting only PI3K/AKT signaling pathway, as a monotherapy, leads to suboptimal activity, supporting the concept that combination of an AKT and androgen signaling axis inhibitor may be preferable for treatment of CRPC.

In conclusion, we show that the novel AKT inhibitor AZD5363 potently inhibits prostate cancer progression at different states in vitro and in vivo. Most important, we show that the combination treatment of AZDS363 plus bicalutamide significantly delays CRPC progression compared with each monotreatment. The high antitumoral activity in vivo combined with an acceptable tolerability profile in this rodent model supports the evaluation of AZD5363 on patients with CRPC in clinical trials. The clinical phase I study for AZD5363 has been launched in December 2010 (44).

Disclosure of Potential Conflict of Interest

B.R. Davies has ownership interest in AstraZeneca as a shareholder. M.E. Gleave has a commercial research grant from AstraZeneca and is a consultant/advisory board member for Astellas. A. Zoubeidi has a commercial research grant from AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: C. Thomas, F. Lamoureux, C. Crafter, B.R. Davies, M.E. Gleave, A. Zoubeidi

Development of methodology: C. Thomas, F. Lamoureux, S. Kim, Daksh Thaper, M.E. Gleave, A. Zoubeidi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Thomas, F. Lamoureux, C. Crafter, B.R. Davies, L. Fazli

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Thomas, F. Lamoureux, C. Crafter, B.R. Davies, E. Beraldi, L. Fazli, M.E. Gleave

Writing, review, and/or revision of the manuscript: C. Thomas, F. Lamoureux, C. Crafter, B.R. Davies, M.E. Gleave, A. Zoubeidi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Thomas, F. Lamoureux

Study supervision: C. Thomas, F. Lamoureux, A. Zoubeidi

Acknowledgments

The authors thank Eliana Beraldi, Virginia Yago, Igor Moskalev, Darrell Trendall, Mary Bowden, and Estelle Li for their technical assistance.
Grant Support
This work was supported by AstraZeneca (A. Zoubiendi and M.E. Gleave), Prostate Cancer Canada (A. Zoubiendi), Prostate Cancer Foundation-USA & Michael Smith Foundation for Health Research (A. Zoubiendi), Deutsche Forschungsgemeinschaft German Research Foundation grant TH 1482/2-1 (C. Thomas), and l’Association pour la Recherche sur le Cancer (F. Lamoureux).

References


44. Stockman P. Assessment of the safety, tolerability and potential anti-cancer activity of increasing doses of AZD5363 in different treatment schedules 2010. Available from: http://clinicaltrials.gov/show/NCT01226316
Molecular Cancer Therapeutics

Synergistic Targeting of PI3K/AKT Pathway and Androgen Receptor Axis Significantly Delays Castration-Resistant Prostate Cancer Progression In Vivo

Christian Thomas, Francois Lamoureux, Claire Crafter, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-13-0032

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2013/08/21/1535-7163.MCT-13-0032.DC1

Cited articles
This article cites 43 articles, 19 of which you can access for free at:
http://mct.aacrjournals.org/content/12/11/2342.full.html#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
/content/12/11/2342.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.