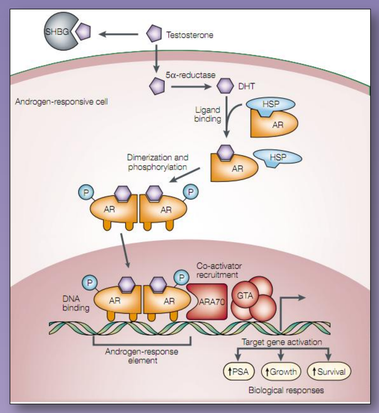
Examining the Efficacy of Novel Androgen Receptor Modulators of Castrate-Resistant Prostate Cancer.

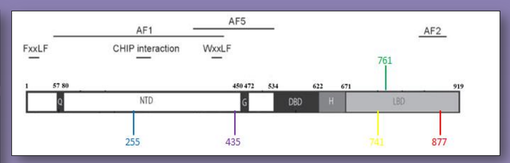
Dominic Jones[1], Daniel O'Neill[2], Fadhel Shaheen[2], Craig Robson[2] and Luke Gaughan[2]

[1] 092515466, Genetics, dominic.jones@newcastle.ac.uk. [2]Northern Institute for Cancer Research.

Introduction

Prostate cancer (CaP) is the leading cause of male-associated deaths in the western world with 10,000 deaths/year in the UK alone. The androgen receptor (AR) is a nuclear hormone receptor transcription factor that is activated by circulating androgens to drive prostate cell transformation. Current therapies attempt to inactivate the receptor by reducing androgens or inhibiting AR ligand-binding (termed anti-androgens).





Mutation	Altered Characteristics
wtAR	n/a
AR_{T877A}	Hydroxyflutamide acts as an agonist
AR_{W741C}	Casodex (AstraZeneca) acts as an agonist
AR_{E255K}	High nuclear localisation
AR_{R761K}	Not understood
AR_{W435L}	Increased AR N/C terminal interaction

Figure 1. AR signalling cascade and structure. (Left panel) Upon DHT binding, AR translocates to the nucleus and binds androgen response elements (AREs) in DNA and activates transcription of androgen regulated genes. (Upper right panel) Domain structure of the AR with indicated mutations used in the study and their effect on AR activity (Lower right panel)

Although initially effective, the cancer ultimately recurs in a more aggressive form, termed castrate-resistant prostate cancer (CRCaP), that is untreatable and fatal. Importantly many CRCaPs retain AR dependency; up to 30% of CRCaP patients acquire AR mutations that facilitates receptor activity to drive cancer cell proliferation. New AR-targeting therapies for CRCaP are therefore required to repress wild type and mutant isoforms of the receptor.

A novel AR down-regulator (ARD1) developed by AstraZeneca has shown promise in cell-line models of CRCaP by destabilising the receptor and reducing cell growth. The aim of this study was to establish the effect of ARD1 on the activity of 5 AR mutants commonly seen in CRCaP patients.

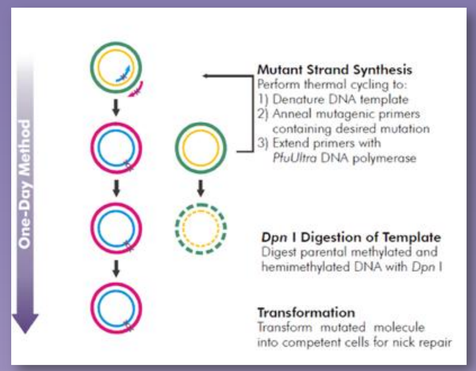
Methods

- **Site directed mutagenesis**. Mutations E255K, W435L, W741C, R761K and T877A were introduced into the pCMV-FLAG-AR expression vector using the QuickChange Site-Directed Mutagenesis Kit (Stratagene).

- **AR protein analysis**. PC-3 cells were transfected with the various AR constructs (+/- MDM2) for 24 hours prior to treatment with DMSO or ARD1 for 24 hours and then subject to Western analysis using anti-AR and –α-tubulin antibodies.

- **AR activity analysis**. PC-3 cells were transfected with various AR constructs and an AR-responsive luciferase reporter for 48 hours prior to treatment with DMSO or ARD1 for 24 hours and then subject to relative luciferase analysis.

Results



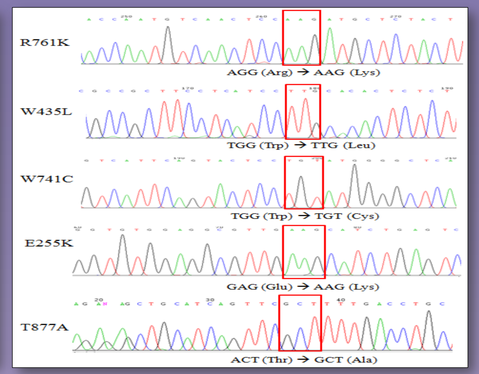


Figure 2. Generation of AR mutants. A) Overview of QuickChange Site-Directed mutagenesis method. B) Chromatograms of AR mutant sequences. Highlighted with a red box is the mutated codon, with the specific base change shown below each chromatogram.

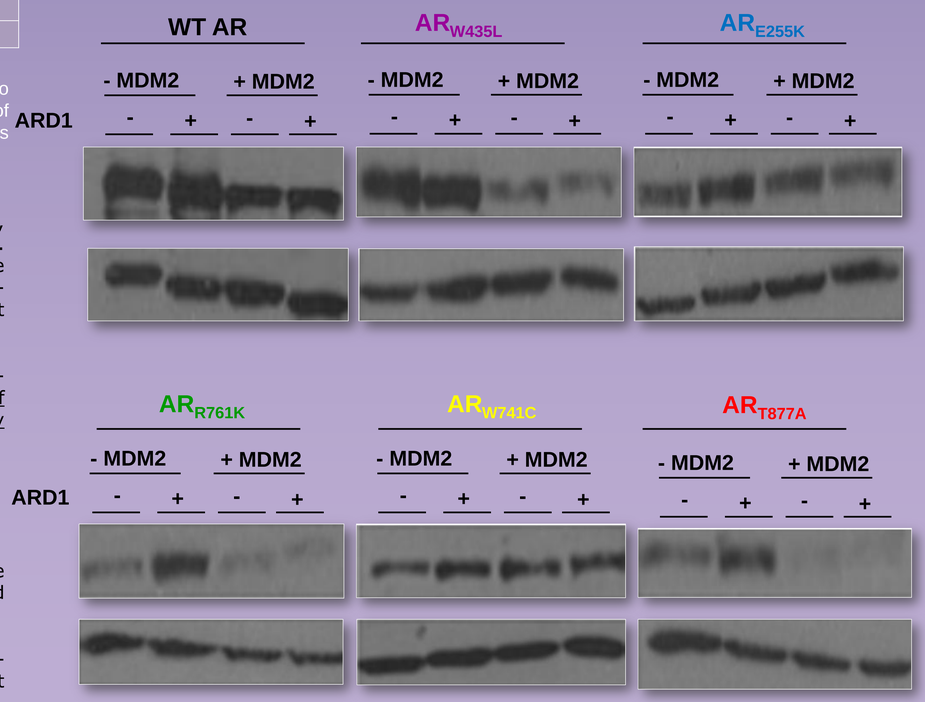


Figure 3. Effect of ARD1 on wild-type and mutant AR protein levels. PC3 cells transfected with either wild-type or mutant AR were treated for 12 hours with ARD1 and then subject to Western analysis. The AR E3 ubiquitin ligase enzyme MDM2 was included as a positive control for AR destabilisation.

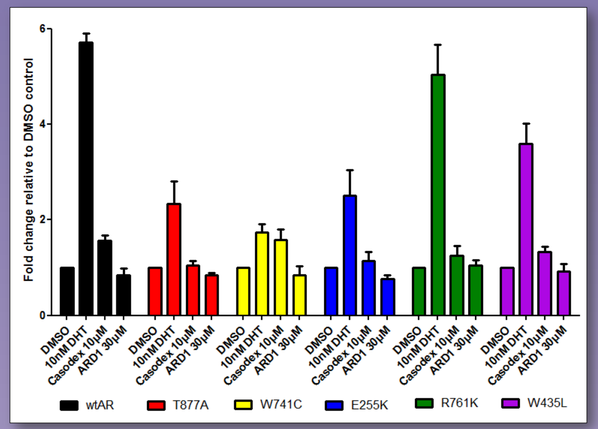


Figure 4. ARD1 is not an AR agonist in PC3 cells. Wild-type and mutant AR activity assessed by luciferase assays post 24-hour DHT, casodex or ARD1 treatment.

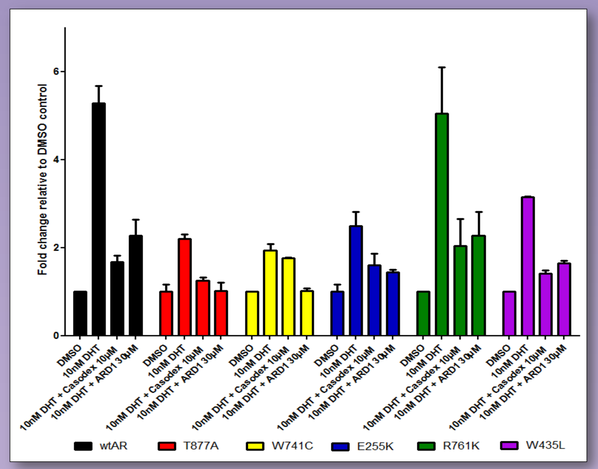


Figure 5. ARD1 reduces DHT-stimulated AR activity in PC3 cells. Wild-type and mutant AR activity assessed by luciferase assays post 24-hour DHT, DHT+ casodex or DHT+ ARD1 treatment.

Conclusion

- 5 AR mutants were successfully created by site-directed mutagenesis to mimic receptor variants identified in CRCaP patients
- All 5 mutants were successfully overexpressed in the PC3 CaP cell-line, but, like wild-type receptor, were not targeted for degradation by ARD1
- Importantly, ARD1 is not an agonist for the AR mutants and attenuates DHT-stimulated activation of the AR suggesting this new anti-androgen may be effective in CRCaP patients with mutated AR