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<b>14. ABSTRACT</b> Growth and development of the prostate is highly dependent on androgen, and aberrant androgen and androgen receptor (AR) signaling is the key driver in the pathology and progression of prostate cancer. Blocking androgen synthesis and inhibiting AR function is the first line of therapy for prostate cancer patients. Although this approach is initially effective in suppressing disease progression, castration resistant tumors eventually develop resulting from reactivation of AR activity. A novel approach to overcome resistance to therapy is to identify selective AR modulators (SARMs) that inhibit the expression of genes promoting tumor growth but enable the expression of genes for differentiation. We hypothesize that functionally distinct set of genes have different promoter signature marks that are recognized by AR. Re-testing and validation of several hits obtained from a high-throughput promoter-dependent compound screen of FDA approved drugs identified a lead compound that had a differential effect on AR promoter-element recognition in transient transfection assays and in chromatin precipitation assays, and in the expression of select AR target genes associated with proliferation and differentiation. Cell proliferation assays indicate that the compound is able to inhibit AR-dependent cell growth. Our discovery of this compound in an unbiased screen for SARMs provides proof-of-concept that drugs may be developed that differentially target promoter elements that distinguish pro-proliferation from pro-differentiation genes. Further optimization of transfection protocol and multiplexing of reporters for the high-throughput promoter screen was performed. A second high-throughput screen was done with a 1.6% hit rate that yielded 26 candidate compounds to be tested for secondary assay validation and mechanism of action analysis.					
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# SELECTIVE GENE REGULATION BY ANDROGEN RECEPTOR IN PROSTATE CANCER

## INTRODUCTION

Androgens mediate their biological effects by binding to the androgen receptor (AR), which functions as a ligand inducible transcription factor. Growth and development of the prostate is highly dependent on androgen and aberrant androgen and AR signaling is the key driver in the pathology and progression of prostate cancer (1). Thus, the first line of therapy for prostate cancer patients consists of blocking androgen synthesis (i.e., by abiraterone) and inhibiting AR function with antagonists (i.e., enzalutamide) (2). Although this approach is initially effective in suppressing disease progression, castration resistant tumors eventually develop resulting from reactivation of AR signaling (1-4). Androgen receptor reactivation can arise from AR amplification, mutation, and altered coregulator interactions, all of which have been shown to be mechanisms underlying recurrent AR activity and formation of castration-resistant prostate cancer (CRPC) (3). Current prostate cancer therapies are circumvented in CRPC (3,5) and there is an outstanding need to identify and develop anti-tumor drugs effective in conditions where AR is reactivated, overexpressed or mutated. In this project, our goal is to identify selective AR modulators (SARMs) that inhibit the expression of genes promoting tumor growth but enable the expression of genes for differentiation. This strategy may identify SARMs that confer selective gene regulation that can reduce resistance and may be useful as an adjuvant therapy against AR reactivation.

## BODY

The grant outlined 3 tasks in the Statement of Work:

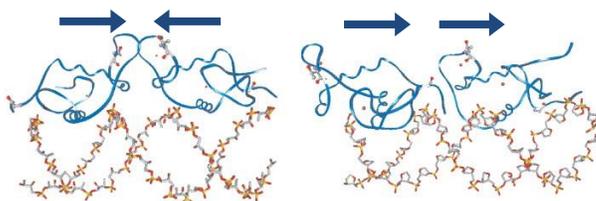
Task 1: Examine the biological effects of the mutant ARs using cell based assays and xenografts

Task 2: Compare the gene expression programs differentially regulated by the mutant ARs

Task 3: Identify molecules that alter AR activity in a promoter-specific manner.

Task 1 and Task 2 were initiated by Dr. E. Lapensee who left for a permanent position. The fellowship transferred to me in November 2012, and I primarily focused on Task 3 in order to validate and characterize primary hits from the promoter-dependent high-throughput screen, as described below.

**Pilot screen.** AR regulates gene transcription by directly binding to DNA or by protein-protein interactions. Hormone response elements bound by AR can be classified as canonical androgen response elements (cARE) composed of 6 bp inverted repeats shared with other steroid receptors or selective AREs (sARE), which are 6 bp direct repeats and selectively bind AR (Fig. 1) (6,7).



**Fig. 1 – Consensus vs. selective AREs**

The AR DNA binding domain dimerizes in a head-to-head fashion on inverted repeats but head-to-tail on direct repeats (modeled from x-ray crystal data). The different configuration imposed by the DNA element exposes different AR surfaces to coregulators.

Studies have shown that allosteric changes in AR conformation may be influenced by the bound DNA sequences (8). Similarly, gene recognition by AR also depends on promoter context, cell type and development or disease stage. Genome wide analysis of AR binding sites has shown that genes regulated by AR in CRPC are distinct from those in normal cells or early disease (9). Mutant ARs can direct different gene regulatory programs and, in some cases, have gained preference for activating cAREs over sAREs (10-12). Moreover, mutations in AR that eliminate binding to sAREs affect the reproductive system and result in reduced fertility in male mice suggesting that AR target genes involved in differentiation rely more on sAREs (13). Taken together this suggests that the sequence and context of AR binding sites has an important role in gene-specific transcriptional control. Further, compounds that interact with different AR domains may influence promoter choice and DNA-binding capacity. Based on this rationale, we developed a high-throughput screen to identify compounds with a differential effect on AR activity dependent on promoter elements. A similar approach succeeded in identifying glucocorticoid receptor modulators (14).

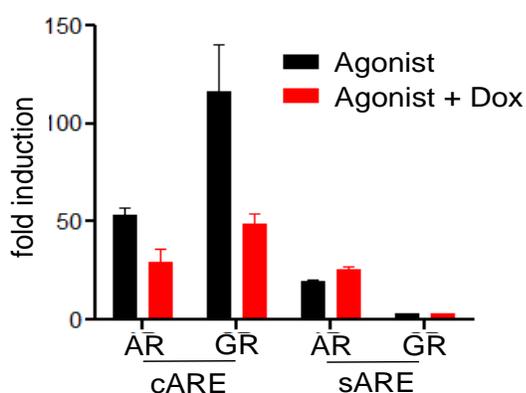
Differential AR activation was assessed using transfected fluorescent reporters driven by multimerized cAREs, sAREs or a PSA promoter in HeLa cells stably expressing AR (HeLa-A6). Screens were performed in saturating levels of the synthetic androgen R1881 to envision promoter activity rather than ligand competition, and optimized for maximal separation of activation vs. AR antagonist inhibition. The pilot screen included 2500 compounds in the Spectrum FDA-approved library and drugs used in NIH clinical trials. Of these compounds, 8% suppressed AR-driven promoters by >75%, and about 3/4 of those suppressed cAREs and sAREs similarly. As an indication of screen validity, known anti-androgens were identified in the screen. To view selectivity, compounds that suppressed one ARE by more than 75% but the other by less than 25% were tallied (Fig. 2). There were 22 cARE-selective compounds that strongly suppressed cARE and had minimal effect on sARE activity. Dose response assays identified 5 cARE-selective compounds with acceptable inhibition curves and potencies to proceed to secondary assays.



**Fig 2.** Venn diagrams show overlapping sets of drugs that strongly suppress one ARE type but have little effect on the other.

**Validation of primary screen hits in transient transfection luciferase assays.** Fresh powder samples of the 5 lead compounds were used to confirm compound activity in transfection assays using cARE, sARE and PSA promoters driving luciferase reporters. Transfection assays were done in robust and easily transfected HeLa-A6 and CV-1 fibroblasts, in a normal prostate epithelial cell line RWPE (AR null), and in several PCa cell lines that included LAPC4 (wt AR), LNCaP (promiscuous AR-T877A), VCaP (high levels of wt AR), and PC-3 (AR null). HeLa and HeLa-A6 cells were extremely sensitive to even very low doses of compounds. The PCa cell lines, LNCaP, VCaP and LAPC4, had very low transfection efficiencies. Androgen-induced

luciferase activity was too low to yield informative results in determining any differential effect. The differential effect of the compounds was also not observed in PC-3 cells or RWPE cells transfected with AR, where the compounds activated both cARE and sARE. However, in CV-1 cells transfected with AR or GR, one of the five hit compounds, doxorubicin (dox), showed a clear differential effect of suppressing cAREs but not sAREs in promoter-luciferase assays. CV-1 cells were also transfected with the glucocorticoid receptor (GR) to test receptor specificity and dependence, and AR/GR chimeras to determine domains that mediate the selective effect. As shown in Fig 3, dox inhibits cARE but not sARE activity in the presence of AR or GR. Similar cARE selective suppression was observed regardless of which AR/GR chimera was used. This suggests that the selective effect of dox is dependent on the sequence of the promoter and that it may intercalate into DNA elements in a sequence-selective manner that may result in differential promoter recognition by AR.

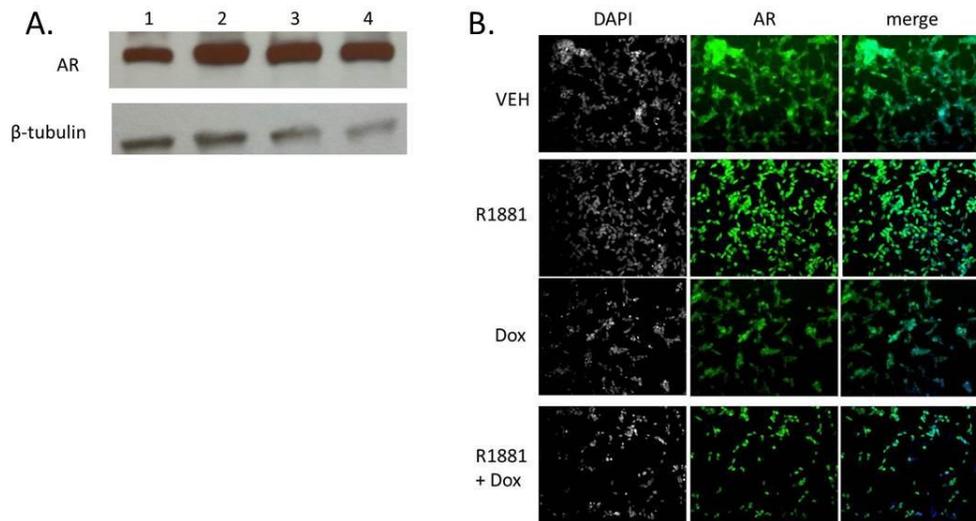


**Fig. 3 – Dox suppresses activation of cAREs but not sAREs.** CV-1 cells were transfected with AR or GR expression vectors and cARE or sARE reporters, and treated with R1881 (AR) or dexamethasone (GR), +/- 0.4  $\mu$ M dox. Dox represses either receptor on cAREs but stimulates AR-specific induction of sAREs.

Doxorubicin is an anthracycline drug that is widely used as a chemotherapeutic agent with a wide spectrum of antitumor activity. Although dox remains one of the most effective chemotherapeutic agents, its mechanism of action is not fully understood. Dox is known to intercalate into DNA and disrupt topoisomerase II action resulting in DNA double strand breaks. This activates the DNA damage response (DDR) to repair the DNA break, and when repair is not successful, apoptosis is initiated (15). Dox increases the expression of tumor suppressor genes p21 and p53 via unknown mechanisms. Dox also generates free radicals that can damage cell membranes, DNA and proteins, which triggers the apoptotic pathway (16). These actions of dox are regarded to be the underlying mechanisms for its anti-cancer activity. However, recent reports show that dox regulates transcription by enhancing nucleosome turnover or histone eviction at promoters and this occurs independent of its ability to cause DNA strand breaks and initiating DDR. (17,18). X-ray crystallography, biochemical data and computer modeling indicate that dox binds to DNA with some sequence selectivity with preferential binding to alternating purine-pyrimidine tracts and affinity affected by neighboring bases. Dox provides proof-of-concept that our transcription based screen detects differences between AREs that model promoter signatures differentially used by AR targets and genes involved in AR signaling.

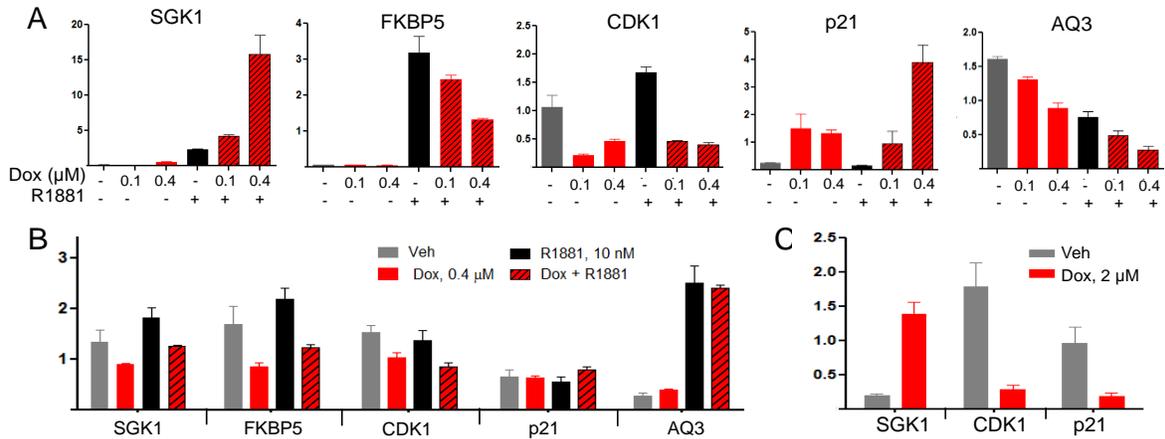
**Effect of compound on endogenous gene expression.** To eliminate the possibility that any differential effect of dox on gene expression may be due to changes in AR expression or localization, we determined the effect of dox on AR mRNA, protein and nuclear localization in

LNCaP cells. Dox did not change AR mRNA expression (not shown) or androgen-induced protein levels (Fig. 4A), and did not alter androgen-induced nuclear localization (Fig. 4B).



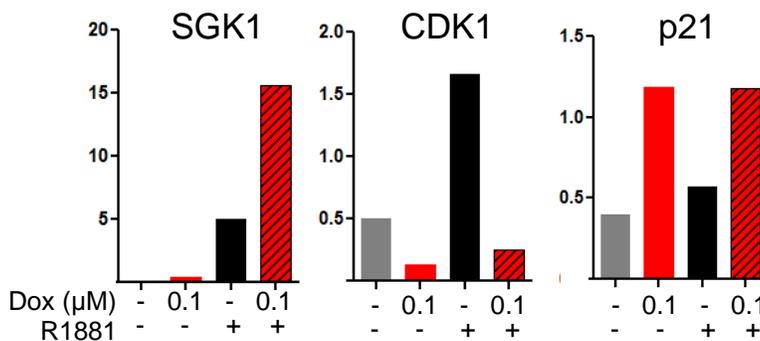
**Fig. 3 – Dox does not affect AR expression.** A. Western blot analysis for AR and  $\beta$ -tubulin (loading control) in LNCaP cells treated with 1) vehicle (VEH), 2) 10 nM R1881, 3) 0.1  $\mu$ M Dox + R1881, and 4) 0.3  $\mu$ M Dox + R1881 cells for 24h. B) Immunofluorescent staining of nucleus (DAPI) and AR in LNCaP cells treated with VEH, 10 nM R1881, 0.4  $\mu$ M Dox and R1881+ Dox.

To determine if the differential effect of dox occurs in natural promoters, particularly those that differ in activity in normal vs. cancer growth, we determined the effect of dox on select AR target genes in prostate cell lines. Since we were interested in inhibiting AR mediated cell proliferation, we looked at the effect of dox on the expression of AR target genes involved in cell cycle regulation. Cyclin dependent kinase 1 (CDK1) is an androgen regulated gene (19) that functions in the S phase of the cell cycle and promotes entry into mitosis (20). Interestingly, CDK1 has been shown to phosphorylate AR to increase its stability (21). The cell cycle regulator p21 is an androgen induced gene that functions as a tumor suppressor by inhibiting CDK activity (22). In LNCaP cells (Fig. 5A), dox had an anti-proliferative effect by inducing p21 expression and repressing androgen-induced expression of CDK1. Dox had varied effects on AR target genes associated with differentiation where it repressed expression of FKBP5 and AQ3, and induced expression of SGK1. For these genes, binding sites for dox or AR are unclear. However, sARE-like elements are essential for AR dependent regulation of SGK1 since male mice that cannot recognize sAREs fail to express SGK1 (13). Gene expression was also examined in normal prostate epithelia using RWPE cells transduced with wt AR (RWPE-AR) to determine the effect of dox in a more normal background (Fig. 5B). In RWPE-AR cells, dox effects were modest and mostly suppressive. In contrast, in AR null PCa cells (PC-3), dox strongly induced SGK1 and repressed CDK1 as in LNCaP but repressed p21 (Fig. 5C). Taken together, dox exhibits gene- and cell-specific effects that may occur via AR as well as through AR-independent mechanisms. In addition, the magnitude of dox response is more pronounced in PCa than in nonmalignant prostate cells.



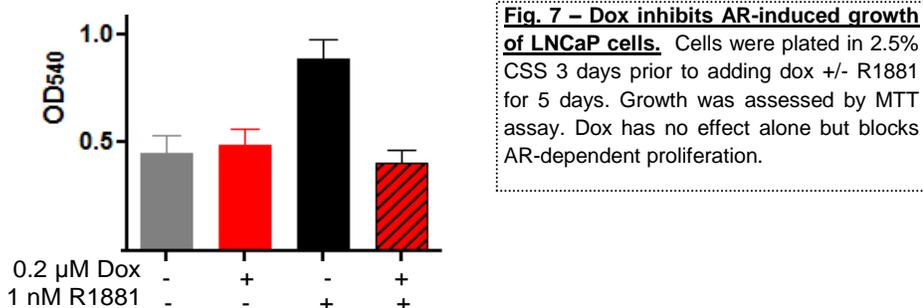
**Fig. 5 – Dox effects are gene- and cell-specific.** Endogenous gene expression was assayed by qRT-PCR of RNA from: **A) LNCaP cells**, **B) RWPE-AR cells** and **C) PC-3 cells**. Cells were plated in 2.5% charcoal stripped serum (CSS) for 3 days, and then treated for 24 hrs with 0.1 or 0.4 μM dox alone or with 10 nM R1881. qPCRs were normalized to GAPDH.

To determine if the differential effect of dox on gene expression occurs primarily at the level of transcription, we measured the levels of nascent RNA transcripts. This was accomplished by labeling newly transcribed RNA with bromouridine (BrU), capturing the BrU-containing RNA with anti-BrU antibodies conjugated to magnetic beads and measuring nascent transcript levels by qPCR. We treated LNCaP cells with R1881 and dox for 24 hours and labeled with BrU for one hour before collecting cells for RTqPCR. Results showed that nascent transcript levels at 24h is consistent with stable mRNA levels, indicating that dox has an early effect on transcription (Fig. 6). This indicates a novel mechanism of action of this drug and a proof-of-concept that distinct AR DNA elements may be differentially recognized by compounds that may then be used to confer gene-selective effects.

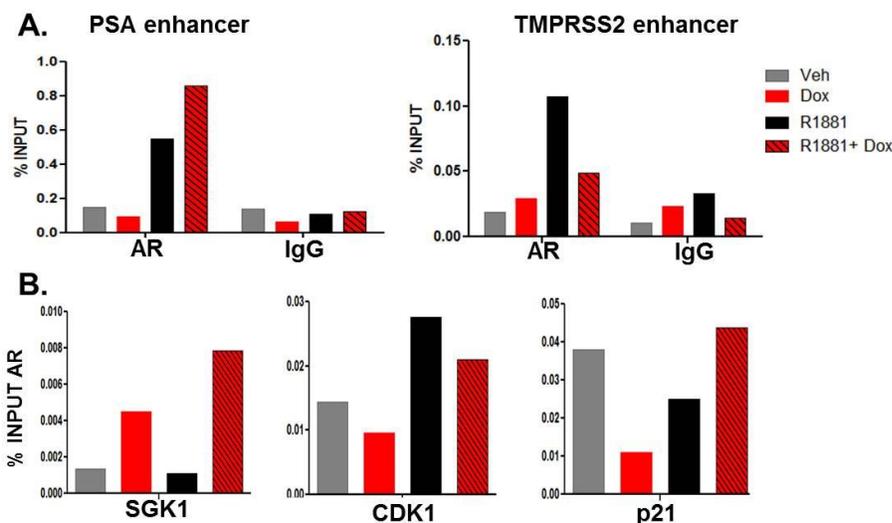


**Fig. 6 – Dox selectively targets genes at an early step in transcription.** LNCaP cells were starved in 2.5% CSS 3 days before treatment for 24 hrs with 0.1 μM dox, 1 nM R1881 or both. 2 mM Bru was added for 1 hr, Bru-RNA isolated and qRT-PCR performed.

**Effect of compound on cell growth.** To determine the effect of dox on AR-driven biology, we measured the effect of dox on cell viability and growth rate by MTT assay. At low dose, dox did not alter LNCaP growth but blocked AR-induced proliferation (Fig. 7). These results fulfill one of the objectives of our screen, which is to identify compounds that block androgen induced cancer-cell proliferation.



**Effect of compound on AR recruitment to target genes.** Using chromatin immunoprecipitation (ChIP) assay, we determined whether the effect of dox on gene expression can be seen at the level of AR recruitment (Fig. 8). ChIP was done after 4 hrs of dox +/- R1881 treatment using an antibody (N20) targeted against the N terminal domain of AR. Effect of R1881 treatment on AR recruitment was confirmed on known AR binding sites (ARBS) at the PSA and TMPRSS2 enhancer where we found an increase in AR recruitment with R1881 that is several fold higher than the IgG background levels. Interestingly, dox increased AR recruitment at the PSA enhancer but decreased R1881-dependent AR association at the TMPRSS2 enhancer (Fig. 8A). Results of a pilot experiment (Fig. 8B) show that for some of the genes we tested, the effect of dox on gene expression follows a trend that is parallel with its effect on gene expression (Fig. 8b). Dox increases AR recruitment to a previously identified ARBS (23) located at -1.1 kb relative from the transcription start site (TSS) of the SGK1 gene. For CDK1, dox leads to a decrease in AR association at an ARBS found in the CDK1 intron (+141 relative from TSS) (24). These effects of dox on AR association follow a trend that is consistent with that seen on SGK1 and CDK1 gene expression levels. However, for p21 the effect of dox on AR recruitment at an ARBS located at the promoter is only consistent with gene expression results from the R1881 + dox treatment group. Differences between the effect of dox on gene expression versus AR recruitment may be attributed to: 1) differences in the time points when gene expression (24 hrs after hormone/drug treatment) and ChIP (4 hrs after hormone/drug treatment) analyses were done, 2) more complex effect of dox on gene regulation that occurs beyond AR recruitment, or 3) context dependent effect of the surrounding chromatin environment.



**Fig. 8 – Dox affects AR recruitment to AR target genes in LNCaP cells.** Cells were plated in 10% FBS for 2 days then starved in 10% CSS for 2 days prior to adding 0.25  $\mu$ M dox, +/- 10 nM R1881 for 4 hours. **(A)** ChIP was done using 10  $\mu$ g of sheared chromatin and 5  $\mu$ g N20 antibody targeted against the AR N-terminal domain, or 5  $\mu$ g IgG. **(B)** Effect of dox on AR recruitment was determined by N20 antibody ChIP followed by qPCR targeted against ARBS found in the SGK1, CDK1 and p21 genes. The effect of dox on AR recruitment follows a trend consistent with gene expression levels.

**Optimization of transfection assays for compound screening.** In validating the hits from the pilot high-throughput screen, we optimized transfection conditions to increase hormone-dependent induction of the cARE and sARE reporters. Different conditions were tested including a comparison between reverse transfection using the Effectene (Qiagen) reagent and forward transfection with XtremeGene (Roche) reagent, comparison of different growth conditions using several serum (full versus charcoal stripped for starvation) concentrations of the growth media, and timing of hormone and compound treatment. The reverse transfection protocol increased R1881-induction levels of cARE-luciferase reporter in HelaA6 cells by 3.4 fold compared to R1881-induction levels obtained with the forward transfection protocol. Starving the cells by growing them in growth media with 2.5% charcoal stripped serum also enhanced hormone-dependent reporter activity. The following is the optimized transfection protocol that yielded highest hormone-dependent activity of the reporter and shortened the protocol by 1 day:

Day 1: Reverse transfection protocol using the Effectene reagent. The premixed complex of transfection reagent and reporters are plated, and cells suspended in growth media with 10% fetal bovine serum (FBS) are then added into plates containing the transfection reagent.

Day2: Twenty hours post-transfection, transfected cells are washed with 1x PBS and media is changed to growth media with 2.5% charcoal-stripped serum to starve the cells. After 8h of starvation, hormone (100 nM R1881) and/or compound is added.

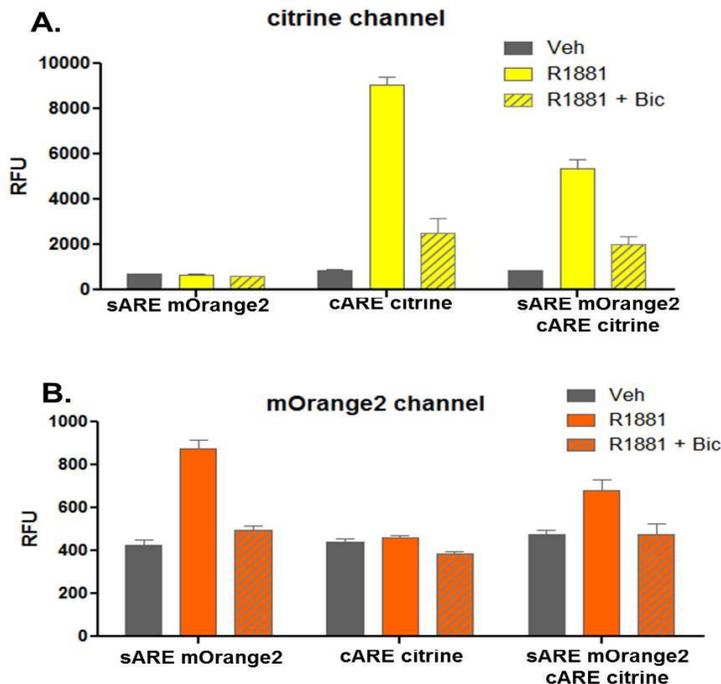
Day3: Reporter activity is measured 22h after hormone treatment.

Since the reverse transfection protocol improved activity, we tested whether we could multiplex cARE and sARE driven fluorescent proteins as originally planned. The sARE or cARE fused to citrine gave 4-5 fold hormone dependent activity. However, the mOrange2 reporters had no activity even with the improved transfection protocol. Sequence analysis of the reporter construct revealed that the poor mOrange2 signal may be explained by an early stop codon generated from cloning the mOrange2 cDNA into the pGL3-ARE backbone (Fig. 9). The early stop codon may interfere with proper translation of the mOrange2 protein so we performed site directed mutagenesis to ablate the early start codon (ATG) and change it to AAT.

AAGCTTGGCATTCCGGTACTGTTGGTAAAGCCA**ac**atg gcc gcc acc **atg** gtg agc aag gcc gag  
gag aat aac atg gcc atc atc aag gag ttc atg cgc ttc aag gtg cgc atg gag gcc  
tcc gtg aac ggc cac gag ttc gag atc gag gcc gag gcc gag gcc cgc ccc **tac** gag  
ggc ttt cag acc gct aag ctg aag gtg acc aag ggt gcc ccc ctg ccc ttc gcc tgg  
gac atc ctg tcc cct caT ttc acc tac gcc tcc aag gcc tac gtg aag cac ccc gcc  
gac atc ccc gac tac ttc aag ctg tcc ttc ccc gag gcc ttc aag tgg gag cgc gtg  
atg aac **tAc** gag gac ggc ggc gtg gtg acc gtg acc cag gac tcc tcc ctg cag gac  
ggc gag ttc atc tac aag gtg aag ctg cgc ggc acc aac ttc ccc tcc gac ggc ccc  
gtG atg cag aag aag acc atg ggc tgg gag gcc tcc tcc gag cgg atg tac ccc gag  
gac ggT gcc ctg aag ggc Aag atc aag atg aag cgc atg aag ctg aag gac cgc ggc cac  
tac acc tcc gag gtc aag acc acc tac aag gcc aag ccc gtg cag ctg ccc ggc  
gcc tac atc gtc **gAc** atc aag ttg gac atc acc tcc cac aac gag gac tac acc atc  
gtg gaa cag tac gaa cgc gcc gag gcc cgc cac tcc acc gcc gcc atg gac gag ctg  
tac aag tct aga ggg ccc tat tct ata gtg tca cct aaa tgc **tag**

**Fig. 9 – Partial DNA sequence of the pGL3 ARE-mOrange2 construct.** DNA sequence in capital letters are part of the ARE promoter. The mOrange2 cDNA sequence is highlighted in yellow with start (atg) and stop (tag) codons in bold letters. The early stop codon is indicated in by the blue box which is part of the sequence (in red letters and underlined) generated from cloning the mOrange2 cDNA into the pGL3-ARE backbone.

The mutated pGL3-sARE mOrange2 construct and pGL3-cARE citrine were used for multiplexing in reverse transfection protocols in a 96-well format. Fluorescent activity of the reporters was measured using the Enspire monochromator-based plate reader. In single reporter transfections, the cARE-citrine (100 ng) reporter gave robust activity with 10.5 fold R1881 induced levels over vehicle and 3.6 fold R1881(100 nM)-dependent induction over the anti-androgen Bicalutamide (Bic, 100 μM) control (Fig. 10A). The sARE-mOrange2 (100 ng) reporter had 2-fold R1881 induction levels over vehicle and 1.4 fold hormone induced levels over Bic control (Fig. 10B). When the cARE-citrine (50 ng) was multiplexed with sARE-mOrange2 (50 ng), R1881 induced citrine activity was 6.4 fold and 2.7 fold over vehicle and Bic control, respectively. The R1881 induced activity of multiplexed sARE-mOrange was 1.4 fold over vehicle or Bic control. Although the mutated pGL3-sARE-mOrange2 now showed R1881-dependent transactivation, the signal did not hold up when the transfections were transformed into the 384-well format.



**Fig. 10 – cARE-citrine and sARE-mOrange2 activity in multiplexed transfection.** Optimized reverse transfection protocol was followed for single reporter (100 ng each of cARE-citrine or sARE-mOrange2) and multiplexed reporter (50 ng each of cARE-citrine and sARE-mOrange2) transfections in a 96-well format. Citrine (A) and mOrange2 (B) fluorescent signal was measured using the Enspire monochromator plate reader.

**High-throughput compound screening.** The optimized reverse transfection protocol was used for screening an additional 7612 compounds from the ChemDiv 100K (3200 compounds), Prestwick (1280 compounds), LOPAC (1280 compounds), MS2400 (960 compounds) and Biofocus natural products (892 compounds) libraries. Compound activity was determined by its ability to inhibit R1881-dependent induction of fluorescent signal from cARE-citrine reporter. Cell toxicity was subsequently measured by Cell Titer Glow (Promega) luminescence assay. Primary screen results showed 124 primary hits or a 1.62% hit rate based on a cutoff score of 70% or greater inhibition of cARE-citrine activity and 50% or less cell toxicity. Out of the primary screen hits, 120 compounds were further tested for dose-response analysis against cARE-citrine and sARE-citrine reporters. There were 109 compounds that showed dose response curves against the cARE-citrine reporter, and 15 out of the 109 compounds showed differential regulation by inhibiting cARE-citrine but not sARE-citrine. This screening method resulted in a hit rate of 0.2% (15 out of 7612 compounds) based on differential regulation of cARE and sARE. One of the lead compounds that came up in this screen has a similar mechanism of action as dox and is a known topoisomerase I inhibitor that intercalates with DNA at strand breaks. This provides further validation that there is a structural difference between the cARE and sARE recognition sequences which affects their recognition by AR, coregulators and compounds that may interact with AR signaling pathway. In addition there is growing interest in the interaction between AR and DNA damage response pathways as targets for prostate cancer therapy (25,26), which emphasizes the significance of further investigation into the mechanism of action and AR pathway interactions as critical in the validation of these lead compounds.

## **KEY RESEARCH ACCOMPLISHMENTS**

- We have tested the lead compounds from a pilot high-throughput screen designed to identify selective AR antagonists. Re-testing and compound validation was done using non-prostate, benign prostate and prostate cancer cell lines.
- We determined the receptor specificity and dependence of the lead compound that showed consistent activity in re-testing and validation assays.
- We have identified a lead compound, doxorubicin, that shows promoter selective effects by suppression of cARE but not sARE activity in luciferase-reporter assays and by differential regulation of the expression of AR target genes associated with proliferation and differentiation.
- We have shown by gene expression analysis that doxorubicin affects the transcription of AR target genes in a gene and cell-context specific manner, with a more pronounced transcriptional effect observed in PCa than normal prostate epithelial cells.
- We have shown that doxorubicin inhibits the AR-dependent growth of PCa cells.
- We have preliminary ChIP data that doxorubicin affects AR recruitment for some genes, in parallel with effects on expression of these AR target genes.
- We have optimized a reverse transfection protocol that reduces high throughput compound screening from 4 days to 3 days.
- We have performed high-throughput screening of an additional 7612 compounds that identified several compounds with similar promoter-selective mechanisms of action as lead compounds identified in the pilot screen.

## **REPORTABLE OUTCOMES**

### **CONCLUSION**

A novel approach to overcome resistance to therapy is to identify selective AR modulators (SARMs) that inhibit the expression of genes promoting tumor growth but enable the expression of genes for differentiation. We hypothesize that functionally distinct set of genes have different promoter signature marks that are recognized by AR. A high-throughput promoter-dependent compound screen identified a lead compound that provides proof-of-concept that promoter elements distinguish pro-proliferation from pro-differentiation genes and that these compounds can affect AR element recognition and AR target gene expression in a cell-dependent manner. In addition, lead compounds identified in the pilot and secondary screen indicate that the interaction between AR signaling and DNA damage response pathways may be feasible targets for prostate cancer therapy.

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