AWARD NUMBER:	W81XWH-19-1-0715
TITLE:	Dynamics of Epigenetic Mechanisms in the Akt Signaling Pathway and Its Influences on Drug Response in Advanced Prostate Cancer
PRINCIPAL INVESTIGATOR:	Dr. Remi Adelaiye-Ogala
CONTRACTING ORGANIZATION:	The Geneva Foundation 917 Pacific Ave #600 Tacoma, WA 98402
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Dynamics of Epigenetic Mechanisms in the Akt Signaling Pathway and Its Influences on Drug Response in Advanced Prostate Cancer

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation 917
Pacific Ave. # 600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective: Preliminary data shows that AKT inhibition (AKTi) is associated with significant anti-tumor effect in multiple models of advanced PCa, which is enhanced when combined with AR targeted therapy. This correlates with increase in expression of some canonically AR-regulated genes, with a prolonged timeline consistent with epigenetic regulation, and with epigenetic changes over the same timeline. This project seeks to characterize how AKTi alters the AR cistrome and identify the mechanism through which this occurs. My approach is to characterize AR cistrome alterations and the broader epigenetic effects combined with a candidate gene approach to test my hypothesis that regulation of KMT2D by AKT mediates the epigenetic effects.

Impact: This project is expected to greatly enhance our understanding of the role of the PI3K/AKT pathway in PCa progression, its interaction with AR activity, and its effect on epigenetic modifications. While this pathway has long been a focus of PCa research, there is renewed interest because of encouraging preliminary clinical results with AKT inhibition. After PARP inhibitors, AKT inhibitors are potentially the next targeted therapy approved for PCa. This study will improve our understanding of the biological effects of this class of drugs and help optimize the use of these drugs in the clinic. At the completion of the proposed projects, results will address the overarching challenges of developing effective treatments that improve outcomes for men with lethal PCa.

15. SUBJECT TERMS
Oncology, Pharmacology, Cancer, Prostate Cancer

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	41	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Background: The PI3K/AKT signaling pathway is essential for cell proliferation, metabolism and survival and

is negatively regulated by PTEN, a tumor suppressor gene frequently lost in prostate cancer (PCa). Preclinical models demonstrate that inhibition of the PI3K/AKT pathway leads to compensatory activation of androgen receptor (AR) signaling, which is supported by clinical data. In breast cancer, another hormone driven, there is compensatory activation of estrogen receptor with PI3K pathway inhibition. This appears to be mediated through the epigenetic regulator, KMT2D. My preliminary data suggest a similar mechanism may link AKT and AR in PCa.

Hypothesis: PI3K/AKT pathway inhibition modulates AR chromatin binding and activity by disrupting regulation of KMT2D by AKT.

Objective: Preliminary data shows that AKT inhibition (AKTi) is associated with significant anti-tumor effect in multiple models of advanced PCa, which is enhanced when combined with AR targeted therapy. This correlates with increase in expression of some canonically AR-regulated genes, with a prolonged timeline consistent with epigenetic regulation, and with epigenetic changes over the same timeline. This project seeks to characterize how AKTi alters the AR cistrome and identify the mechanism through which this occurs. My approach is to characterize AR cistrome alterations and the broader epigenetic effects combined with a candidate gene approach to test my hypothesis that regulation of KMT2D by AKT mediates the epigenetic effects.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Prostate cancer, therapeutic resistance, AKT inhibition, androgen receptors, epigenetic landscape, canonical AR cistrome, non-canonical AR cistrome, KMT2D, H3K27ac and glucocorticoid receptor.

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

The major goals of the projects are:

a. Identify how AKT inhibition alters AR regulome, cofactors and changes in epigenetic landscape that cooperate to drive its activity in various prostate cancer cell lines and PDX models. The initial proposed time to complete experiments to achieve this goal was at the month 20 of the award period. Due to the impact of COVID pandemic and transition to a new institute, the new proposed time to complete this project is at 26 months with No cost extension (NCE). We hope to have achieve this goal/milestone by the next reporting period.

b. Through co-immunoprecipitation of endogenous KMT2D and AKT, determine direct interaction of both protein, production of stable cell lines and PDX organoids engineered to overexpressing KMT2D and knock out KMT2D. Evaluation of KMT2D role in response to PI3K/AKT inhibition. Evaluation of KMT2D on AR and GR following therapy. The proposed time to complete experiments to achieve this goal is at the month 16 of the award period. Due to the lockdown that resulted from the COVID-19 pandemic, only 20% of this goal has been achieved. We hope to have achieve this goal/milestone by the next reporting period.

c. The third goal/ milestone is that we would have, identified how KMT2D mediates the effect of AKT on the epigenetic landscape. In addition, we hoped to have publication of 1-2 peer reviewed papers coming from this project. The proposed time to complete achieve this goal is at the month 36 of the award period with the approved NCE. So far, we have one peer-review publication from results

Specific Aims:

Aim 1 will be focused on performing comprehensive analysis identifying changes in AR cistrome and the broader epigenetic landscape after exposure to PI3K/AKT pathway targeted therapies.

Aim 2 will be focused on performing mechanistic studies to test the hypothesis that PI3K/AKT signaling alters AR activity and cofactor binding through KMT2D.

What was accomplished under these goals? *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

In the prior report, we presented in PCa models, LNCaP (enzalutamide sensitive, AR+, PTEN null and hyperactive PI3K/AKT signaling), LREX (Enzalutamide resistant, AR+, PTEN null and hyperactive PI3K/AKT signaling) and LuCaP 136 (Patient derived xenograft, low enzalutamide sensitivity, AR+, PTEN null and hyperactive PI3K/AKT signaling) various responses to pan AKT inhibitors (ipatasertib) as well as their response to AR antagonist (enzalutamide) or combination of both drugs (**Figure 1A-C**). Furthermore, we found that enrichment of non-canonical AR target genes in models less sensitive or resistant to enzalutamide (LREX and LuCaP 136). Non-canonical gene set was built based on previously identified non-canonical AR target genes (*Wang F, et al. Androgen receptor cistrome in prostate differentiation and cancer progression. Am J Clin Exp Urol 2017;5(3):18-24, Pomerantz MM, et al. The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. Nat Genet 2015;47(11):1346-51*

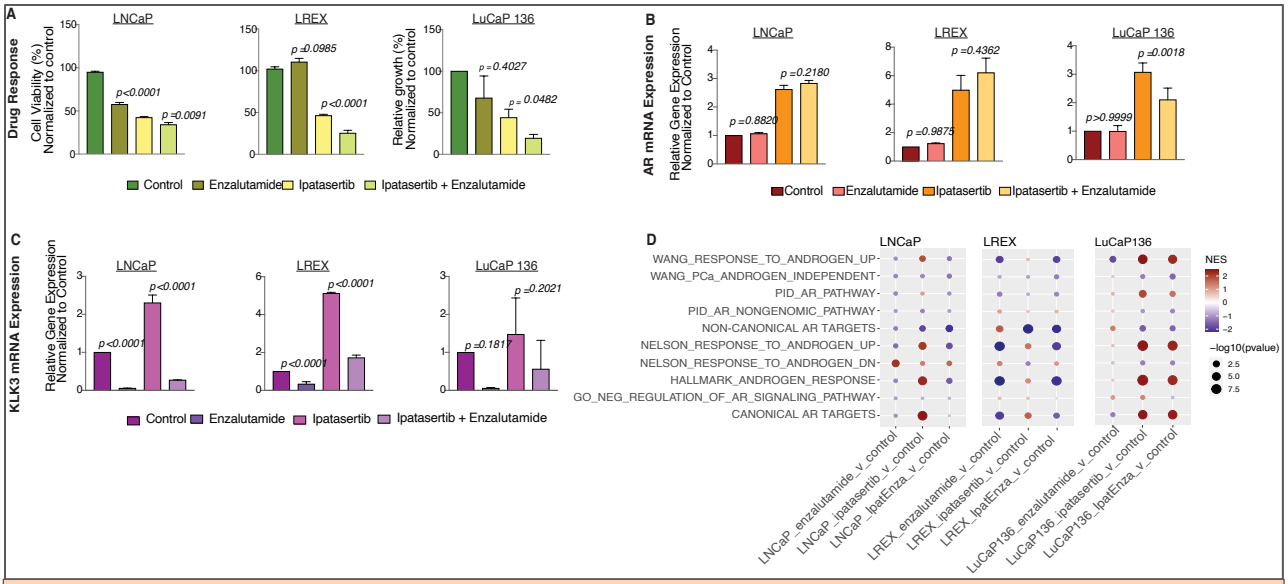

Figure 1: Kinase inhibitor (ipatasertib) increases canonical AR activity and overrides enzalutamide resistance. (A) Bar plots indicating response to ipatasertib, enzalutamide or combination of both drugs in cell lines and patient derived xenograft model of prostate cancer. (B) Gene expression data by qRT-PCR indicates no change in AR expression in enzalutamide treated cells and tumors compared to controls and a significant increase in AR expression following exposure to Ipatasertib. (C) Gene expression data by qRT-PCR shows increase in canonical AR target genes with Ipatasertib and decrease with enzalutamide exposure. (D) Bubble plots from gene set enrichment analysis (GSEA) highlighting differential enrichments in established AR signatures, specifically showing a switch from enrichment of non-canonical AR targets genes to canonical AR targets following kinase inhibition in enzalutamide less sensitive and resistant models. Bar plots are presented as mean +/- SD.

doi10.1038/ng.3419, Chattopadhyay I, et al. Src promotes castration-recurrent prostate cancer through androgen receptor-dependent canonical and non-canonical transcriptional signatures. Oncotarget. 2017;8(6):10324-47). When these models were exposed to pan-AKT inhibitor, ipatasertib, we observed restoration of canonical AR target genes. In combination treatment, there was decrease enrichment of both non-canonical AR target genes influenced by ipatasertib and decrease in canonical AR activity which was associated with response (**Figure 1D**). The data suggested that restoration of canonical AR activity re-sensitized cells to enzalutamide. In parallel performed chromatin immunoprecipitation (ChIP) targeting AR and H3K27ac (marks active sites) followed by next generation sequencing to determine AR binding patterns across treatment conditions. We identified unique AR enriched peaks at active sites associated with enzalutamide resistance (**Figure 2A-B**) and through GSEA of annotated peaks, we found unique regulatory transcription factor targets and biological processes, including regulation of the glucocorticoid receptor (GR) pathway known to be involved in cancer processes such as cell proliferation and differentiation (**Figure 2C**) which was reversed when following AKT inhibition. Previous studies have shown that prostate cancer cells can also bypass AR blockade through induction of other hormone receptors, in particular the GR. These results demonstrated that inhibition of the AKT signaling pathway can block GR activity and overcome GR-mediated resistance to AR-targeted therapy.

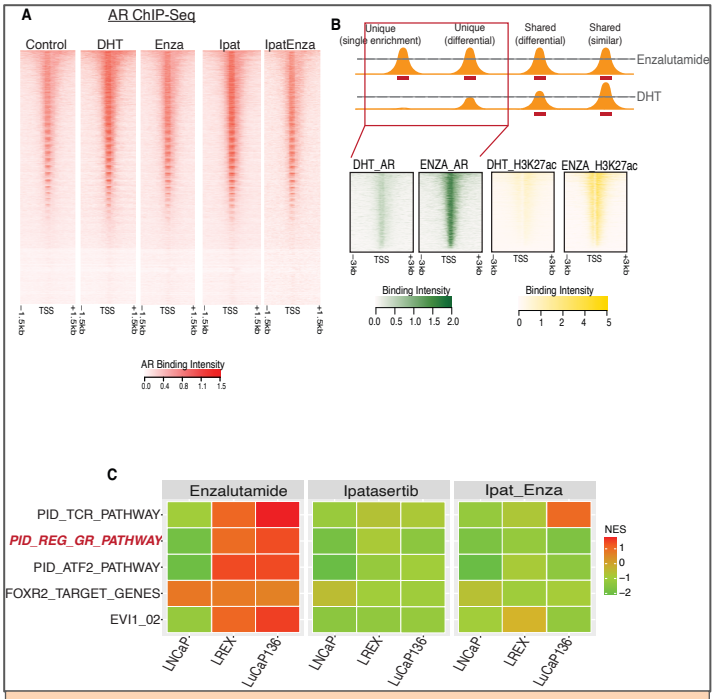

Figure 2: Enriched regulatory targets and biological processes identified in GSEA of gene annotated unique AR bound regions were decreased following kinase inhibition. (A) Heatmap indicates overall AR binding intensity across treatment conditions including DHT in an enzalutamide resistant PCa cell line. (B) Schema presenting how we identify and define enriched AR binding regions unique to enzalutamide resistance (top panel). AR ChIP-seq heatmap (green) shows that unique AR peaks associated with enzalutamide resistance are active as indicated by increased H3K27ac binding intensity (gold) and are significantly decreased or absent in the presence of DHT. (C) Heatmap of some selected enriched biological processes including compensatory pathway, GR and regulatory factor targets associated with gene annotated enzalutamide resistant unique AR peaks.

Since our last report, we have performed an in-depth profiling of the epigenetic landscapes specifically at active sites and including enhancers/super-enhancers associated with PI3K/AKT

signaling and response to drug treatment in advanced PCa. The of this, is to determine active chromatin patterns in the epigenome that is influenced by inhibition of AKT signaling. Epigenetic processes such as histone modifications at cis-regulatory elements can affect gene transcription independent of their orientation or distance via enhancers (*Heintzman, N.D. & Ren, B. Finding distal regulatory elements in the human genome. Curr Opin Genet Dev 19, 541-549)*. H3K27ac has been established as an important mark of enhancers which distinguishes between active and inactive regions (active referring to a positive influence on the expression of proximal genes) (*Creyghton, M.P., et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 107, 21931-21936*), hence, regions with deposits of H3K27ac are often associated with enhanced gene activity. To this end, we performed H3K27ac-ChIP followed by high-through-put next generation sequencing in cell lines and PDX models of advanced PCa. Our data show overall subtle changes in the global binding of H3K27ac across multiple samples across all treatment conditions specifically in models resistant to enzalutamide (LREX & LuCaP 136) (**Figure 3**). Next, we looked at super-enhancers, SEs (a class of regulatory regions with unusually strong enrichment for the binding of transcriptional coactivators) associated with each treatment condition. SEs defined with H3K27ac ChIP-seq experiments and based on ranking orders of SE using ROSE2(*Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell. 2013;153(2):307-19. Epub 2013/04/16. doi: 10.1016/j.cell.2013.03.035*, *Loven J, Hoke HA, Lin CY, Lau A,*

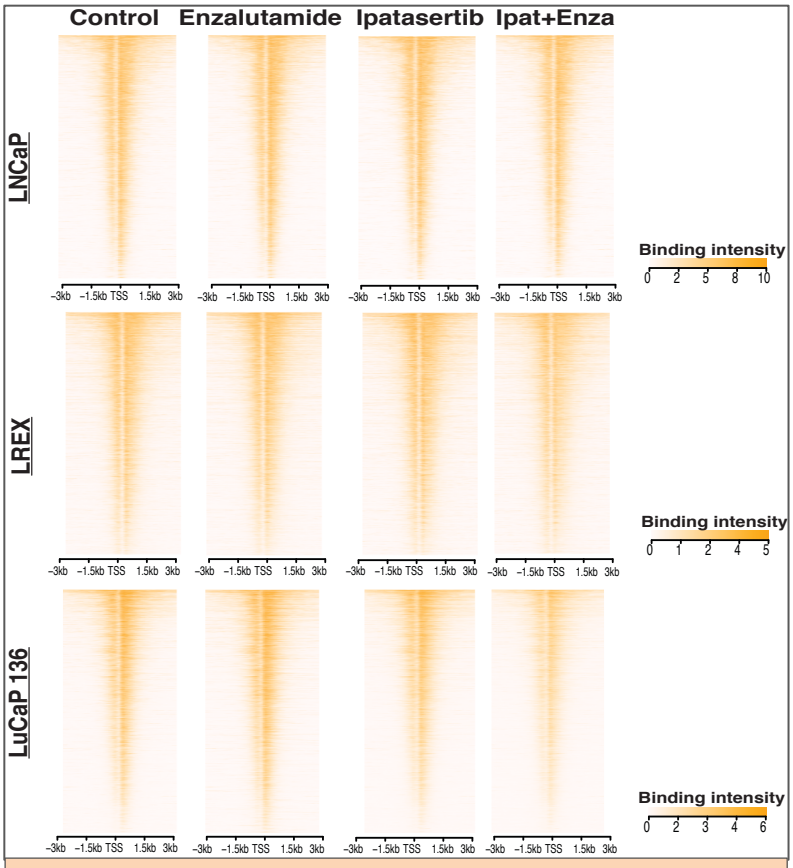

Figure 3: Global H3K27ac binding patterns in multiple models of advanced PCa. Heatmap indicates overall H3K27ac binding intensity across treatment conditions including DHT in enzalutamide sensitive and resistant

Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013;153(2):320-34. Epub 2013/04/16. doi: 10.1016/j.cell.2013.03.036) Interesting, enzalutamide resistant models attracted more SEs when exposed to enzalutamide. However, when these same models were exposed to ipatasertib the abundance of SEs was decreased and this decrease was maintained when ipatasertib was combined with enzalutamide (**Figure 4**). We did not notice any significant change in SEs in

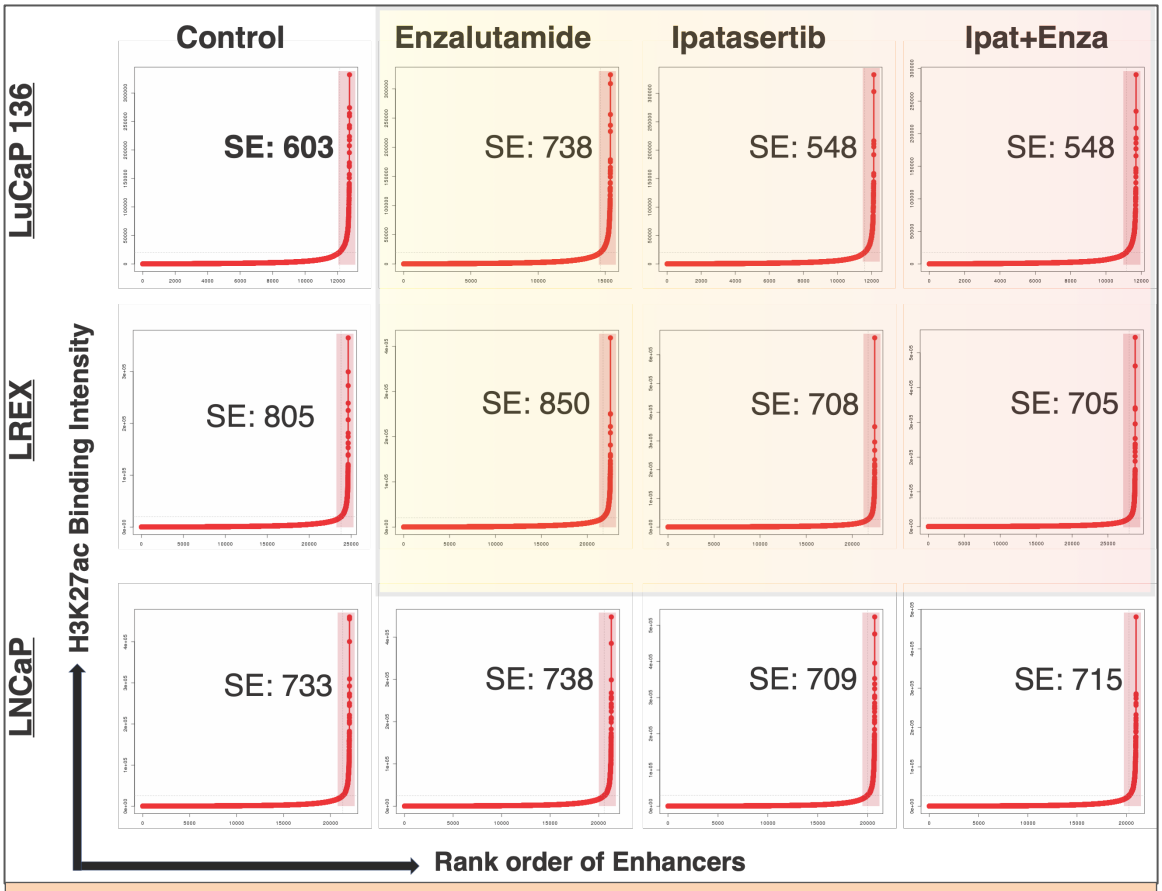

Figure 4: Enzalutamide resistance is associated with increased super-enhancers which is abrogated with the inhibition of PI3K/AKT signaling. Generated plots present ranking order of super enhancers (SEs) across treatment conditions in multiple models of advanced PCa. Highlighted plots indicate models where increased SEs was observed with enzalutamide and decreased with ipatasertib alone or in combination with enzalutamide in resistant models.

enzalutamide sensitive model, LNCaP irrespective of treatment conditions. To understand the epigenetic networks involved, we sought to identify regulatory circuitry associated with enzalutamide resistance and the impact of AKT inhibition by performing analysis of SE-associated TFs in resistant models. For a given SE-associated TFa, the input (in degree) was calculated as the number of all TFs (motifa + motifb + motifc +…motifn) with a motif present in nucleosome-depleted valleys of TFa's SE. The output for TFa was calculated as the total number of SEs (with putative TF target genes; SE1, SE3, SE4 and so on) that had the motif of TFa (out degree). Total connectivity (in + out degree, normalized to 1 representing maximum connectivity

in the sample) predicted the TFs with high connectivity, the 'core' of the regulatory circuitry. We then ranked the identified core TFs based on their gene expression across treatment conditions in our enzalutamide resistant, ipatasertib positive models (**Figure 5A**). Among the top ranked core

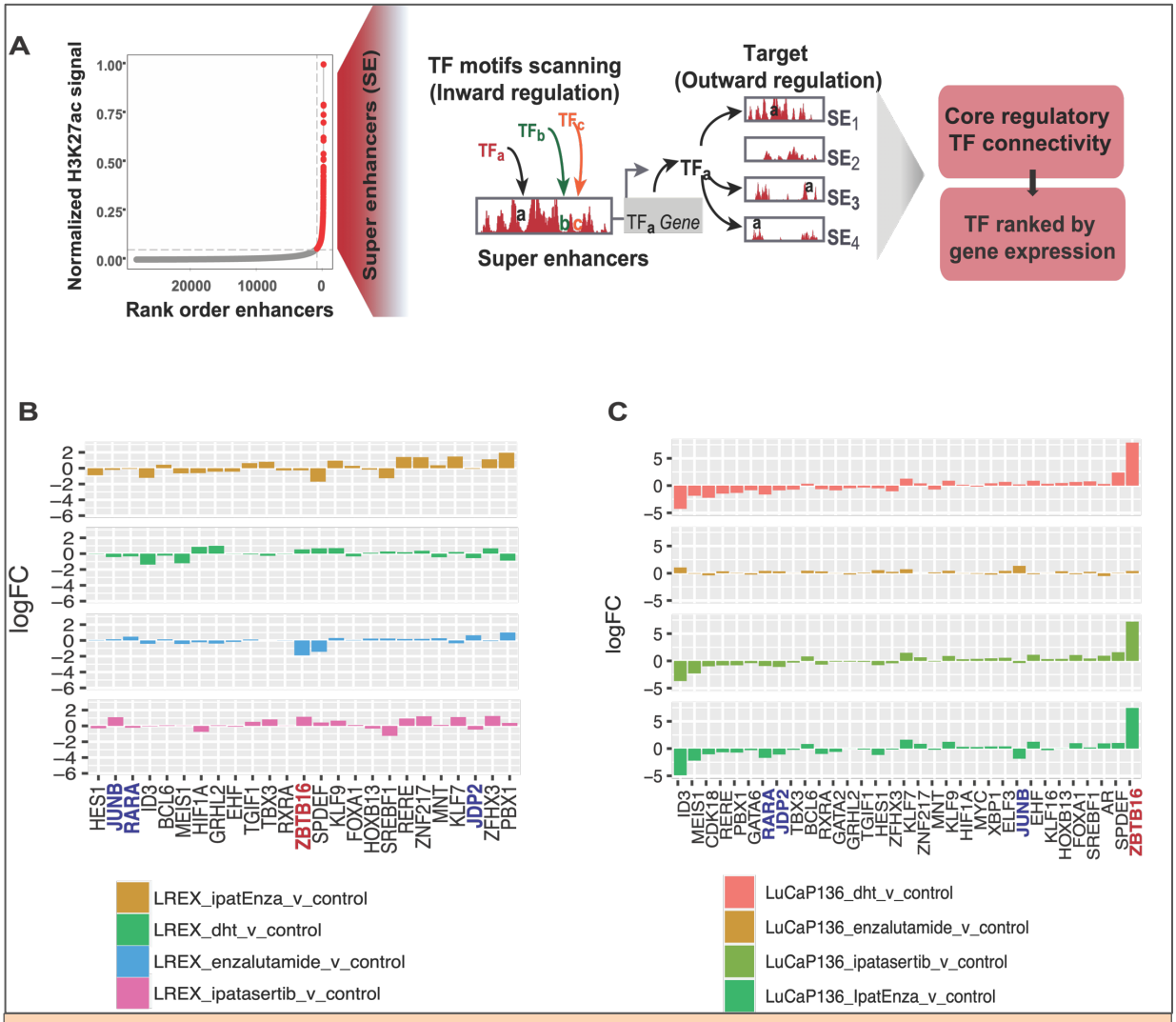

Figure 5: Master regulatory transcription factors prediction identifies master transcription factors associated with enzalutamide resistance. (A) Model for identifying and prioritization of master regulatory transcription factors associated with resistance to enzalutamide. (B-C) Expression of regulatory TFs across treatment conditions in our models of enzalutamide resistant, ipatasertib sensitive PCa.

regulatory TFs identified, expression of non-canonical AR target genes was consistently increased in enzalutamide resistance (*example highlighted in blue)* and decreased with ipatasertib treated as single agent or in combination (**Figure 5B-C**). Consistent with our initial findings, genes associated with canonical AR activity had increased expression with ipatasertib compared to enzalutamide (*example highlighted in red*) (**Figure 5B-C**). This provides us with insight as to how PI3K/AKT signaling may be playing a role in AR cistrome programs and the influence on response to drug treatment. We are conducting more studies to validate these findings in

additional models. In addition, we have ongoing experiments to other histone modifications, H3k27me3, K4me1 & 2, to determine associated post translational modifications that may contribute to this phenomenon.

In specific aim 2, the goal is to test the hypothesis that PI3K/AKT signaling alters AR activity and cofactor binding through KMT2D, a histone methyltransferase. This hypothesis was based on previous studies in breast cancer, where inhibition of the PI3K/AKT pathway increased estrogen receptor (ER) expression and alterations in ER cistrome with enrichment for sites with FOXA1 motifs. It has been reported that AKT phosphorylates KMT2D leading to loss of KMT2D function and its ability to recruit cofactors like FOXA1 and PBX1 for AR transcriptional activity (*Toska, Eneda, et al. "PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D." Science 355.6331 (2017): 1324-1330*). In our studies, we have seen increases in KMT2D mRNA expression following exposure to ipatasertib as a single agent or in combination with enzalutamide (Figure 4A). In addition, we found enriched AR binding around the KMT2D loci following exposure to ipatasertib in a resistant model (See Figure 4B) suggesting an interaction between AR and KMT2D. We plan to proceed with mechanistic studies proposed in aim 2 to gain an in-depth understanding of the link between AKT and KMT2D in prostate cancer, and the role played in alteration of the AR regulome and the broader epigenetic landscape.

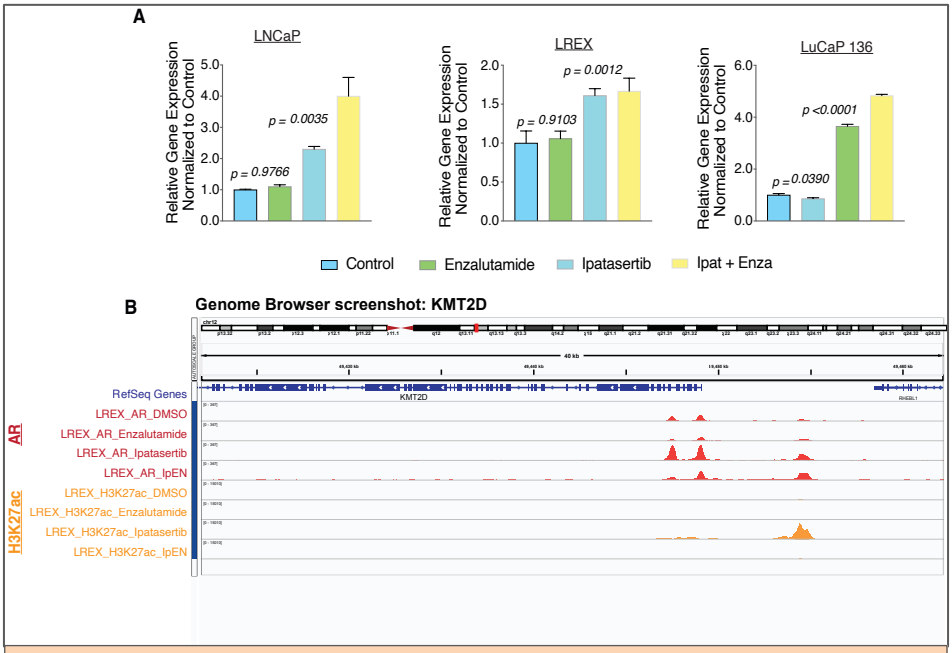

Figure 6: Alterations in KMT2D expression and AR binding following inhibition of the AKT signaling. (A) Bar plots indicate differential expression of KMT2D mRNA expression in all models following exposure to AKT inhibition or in combination with both drugs. (B) Genome browser screenshots show enriched AR binding near KMT2D locus in a resistant prostate cancer models exposed to AKT inhibition

What opportunities for training and professional development did the project provide?
How were the results disseminated to communities of interest?

While at the NCI, I had access to a wealth of resources through the Office of Intramural Training and Education for educational training and career development. I completed course work in Clinical Oncology and Research Mentorship Training. Through this award, I was able to further my training and career development in prostate cancer research by enrolling and completing course work in Translational Research in Cancer Biotechnology and Statistical Analysis of Research Data. I also attended seminars and workshops in Grant/Scientific Manuscript Writing, Scientific Management Training, Diversity in a Multicultural Society, and Becoming a Resilient Scientist. I have had the opportunity to attend and present research work from this project at departmental seminars (NCI), national and international scientific conferences (12th Annual Multi-Institutional Prostate Cancer Program Retreat, Prostate Cancer Foundation 26th Annual Scientific Retreat). These events provided an environment for professional networking through which I found and established collaborations with research scientist, physician scientists and oncologists interested in working together on future projects. This also opened the gate into my transition to a faculty position at the University at Buffalo.
Currently as a junior faculty in the department of Medicine, I have presented results from this project at University of Buffalo- Cancer Research Consortium Seminar. I have also presented this work at the Clinical and Translational Science Institute (CTSI) K Scholar Program seminar.

How were the results disseminated to communities of interest?

Results from this project has been disseminated to the research communities, through peer-reviewed publication, and presentation at scientific meetings and seminars.

What do you plan to do during the next reporting period to accomplish the goals and objectives?

The goal of specific aim 1 is to identify how AKT inhibition alters the AR cistrome, the cofactors and changes in epigenetic landscape that cooperate to drive its activity in various prostate cancer cell lines and PDX models. Currently, we are assessing influence of kinase inhibition on AR cistrome using other PI3K/AKT inhibitors. In addition, we plan to perform ATAC-seq experiments to determine global changes on the epigenome following response to AKT inhibitors

and enzalutamide. We have already access epigenetic changes specifically at enhancers and super-enhancer regions that play a major role in the transcription machinery and cell fate associated with AKT inhibitors and enzalutamide in 3 prostate cancer models. For specific aim 2, we will focus on generating stably transfected cells for mechanistic studies to test the hypothesis, that PI3K/AKT signaling alters AR activity and cofactor binding through KMT2D.

4. IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

The long history of PI3K/AKT pathway inhibitors for the treatment of prostate cancer and other solid tumors has translated to only modest success in the clinic. The pan-AKT inhibitor ipatasertib shows promise in combination with abiraterone plus prednisone for late-stage prostate cancer. Our data demonstrate AKT inhibition of established xenografts leads to increased canonical AR activity which is associated with decreased GR expression (a signaling pathway associated with therapeutic resistance and non-canonical AR activity), and marked anti-tumor activity in models that upregulate GR to induce resistance. These findings have the potential to provide a better understanding of the clinical settings in which GR activity is most critical will help usher in a new target in the prostate cancer therapy armamentarium

What was the impact on other disciplines?

Nothing to report during this reporting period

What was the impact on technology transfer?

Nothing to report during this reporting period

What was the impact on society beyond science and technology?

Nothing to report during this reporting period

5. CHANGES/PROBLEMS:

The PD/PI is reminded that the recipient organization is required to obtain prior written

approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

There are no significant changes in our approach in this project. Minor changes include the use of LuCaP 136 as a replacement for LuCaP 86.9 as a better sustainable model. LuCaP 136 is a patient derived xenograft model that is positive for AR expression, PTEN null and has a hyperactive PI3K/AKT signaling pathway which represents clinical disease.

Actual or anticipated problems or delays and actions or plans to resolve them:

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to impacts of the COVID-19 pandemic and transitioning to new institution, there has been a delay in meeting experiments timelines. Change of institution form NCI, MD to University at Buffalo, NY resulted in a temporary pause on the study in order to submit approval request and initiate subaward at new site. We have re-evaluated the timeline of experiments to address all proposed aims. We anticipated changes in expenditures and cost of services due to these delays and have adjusted for these changes.

Changes that had a significant impact on expenditures

Within this reporting period, we have experienced impacts on experiments timeline due the COVID-19 pandemic as well as transitioning during this period to a new institute. We request and got approved for a no cost extension and have re-evaluated the timeline of experiments to address all proposed aims within the extended period. We anticipated changes in expenditures and cost of services due to these delays and have adjusted for these changes.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

There are no significant deviations, unexpected outcomes or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards and/or selected agents during this reporting period.

Significant changes in use or care of human subjects:
Not Applicable to this research

Significant changes in use or care of vertebrate animals:
Not Applicable to this research

Significant changes in use of biohazards and/or select agents:
There are no significant changes in the use of biohazards and/or selected agents

6. PRODUCTS:

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

Publications, conference papers, and presentations

Remi M. Adelaiye-Ogala, Berkley Gryder, Yen Thi Minh Nguyen, Aian Neil Alilin, Adlai Grayson, Keith H. Jansson, Michael L. Beshiri, Supreet Agarwal, Jose Antonio Rodriguez-Nieves, Brian Capaldo, Kathleen Kelly and David J. VanderWeele. *Targeting the PI3K/AKT pathway overcomes enzalutamide resistance by inhibiting induction of the glucocorticoid receptor*. Mol Cancer Ther. 2020 May 5. doi: 10.1158/1535-7163.MCT-19-0936

Journal publications:

Remi M. Adelaiye-Ogala, Berkley Gryder, Yen Thi Minh Nguyen, Aian Neil Alilin, Adlai Grayson, Keith H. Jansson, Michael L. Beshiri, Supreet Agarwal, Jose Antonio Rodriguez-Nieves, Brian Capaldo, Kathleen Kelly and David J. VanderWeele. *Targeting the PI3K/AKT pathway overcomes enzalutamide resistance by inhibiting induction of the glucocorticoid receptor*. Mol Cancer Ther. 2020 May 5. doi: 10.1158/1535-7163.MCT-19-0936

Books or other non-periodical, one-time publications:

Nothing to report

Other publications, conference papers, and presentations:

Poster Presentation: Remi M. Adelaiye-Ogala, Brian J. Capaldo, Berkley E. Gryder, David J VanderWeele and Kathleen Kelly. *Alterations in AR regulome following response to kinase inhibitors*

Remi M. Adelaiye-Ogala, Berkley Gryder, Yen Thi Minh Nguyen, Aian Neil Alilin, Adlai Grayson, Keith H. Jansson, Michael L. Beshiri, Supreet Agarwal, Jose Antonio Rodriguez-Nieves, Brian Capaldo, Kathleen Kelly and David J. VanderWeele. *Targeting the PI3K/AKT pathway overcomes enzalutamide resistance by inhibiting induction of the glucocorticoid receptor.* *

Website(s) or other Internet site(s):
https://pubmed.ncbi.nlm.nih.gov

Website description: PubMed comprises more than 30 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.

Technologies or techniques:
There are no novel technologies or techniques that has resulted from research activities during this reporting period.

Inventions, patent applications, and/or licenses:
There are no inventions, patent application and/or licenses that have resulted from this research.

Other Products:
Nothing to report for this period.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Remi Adelaiye-Ogala, PhD
Project Role:	PI

Researcher Identifier (e.g. ORCID ID):	ORCID ID: https://orcid.org/0000-0002-6581-7359
Nearest person month worked:	12.00 calendar months
Contribution to Project:	As PI for this study, I perform/supervise all aspects of this project, including tissue dissection, library preparation, sequencing, and data analysis and target validation
Funding Support:	An employee of University at Buffalo, Research Foundation. 1.2 calendar months support by DOD_EIRA

Name:	Brian Capaldo, PhD
Project Role:	Bioinformatician (Collaborator)
Researcher Identifier (e.g. ORCID ID):	ORCID ID: https://orcid.org/0000-0003-1244-8884
Nearest person month worked:	0.60 calendar months
Contribution to Project:	Provides statistical analysis and bioinformatics analysis of next-generation sequencing data
Funding Support:	An employee of the federal government (NIH)

Name:	David J VanderWeele, MD, PhD
Project Role:	Secondary Mentor
Researcher Identifier (e.g. ORCID ID):	ORCID ID: https://orcid.org/0000-0003-4576-5034
Nearest person month worked:	.60 calendar months
Contribution to Project:	Provides guidance and direct aspects of the research that can be effectively applied in the clinic
Funding Support:	An employee of Nonwestern University

Name:	Kathleen Kelly, PhD
Project Role:	Primary Mentor
Researcher Identifier (e.g. ORCID ID):	Scopus ID: 7401769579
Nearest person month worked:	.60 calendar months
Contribution to Project:	Provides primary guidance in experimental design and technical aspects of the research described in this proposal. Assist in abstract/manuscript writing, provide guidance on the progress of this project and promote opportunities for to present at international and national prostate cancer research meetings
Funding Support:	An employee of the federal government (NIH)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

PI accepted a position as Research Assistant Professor in the department of Medicine, Jacobs school of medicine and biomedical sciences which resulted in a change of institutes and a percentage of her support comes from the institute. There is no overlap with support from the DOD.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Organization Name-1: CCR Sequencing Core Facility-Illumina
Location of Organization-1: Center for Cancer Research,
BLDG. 427, Room 1,
NCI, Frederick, MD 21702)
Partner's contribution to the project

Sequencing core facility was actively involved in provide high through-put next-generation sequencing on our RNA and ChIP-DNA samples. Core facility also performed quality control (QC) analysis on samples prior to sequencing.

Organization Name-2: University at Buffalo
Location of Organization-2: Clinical and Translational Research Center, University at Buffalo,
875 Ellicott Street, 6081,
Buffalo, NY, 14203

Partner's Contribution to the project:

Primary site to continue remaining experiments proposed in this project.

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Quad Chart Attached

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

- *Curriculum Vitae Attached*
- *Abstracts Attached*
- *Manuscript Attached*

Curriculum Vitae

Date Prepared: September 15, 2021

Name: Remi M. Adelaiye-Ogala, PhD

Office Address: University at Buffalo
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875 Ellicott Street
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Buffalo, NY 14203

Home Address:

Work Phone: (716) 887-2202

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Personal Email: remi.adelaiye@gmail.com

Place of Birth: Nigeria

USA Status: Permanent Resident

Education

2017	Ph.D.	Cancer Pathology (mentor: Roberto Pili, MD)	Roswell Park Cancer Institute Division, University at Buffalo
2009	B.S.	Biochemistry	State University of New York at Fredonia

Postdoctoral Training

2017-2020	Postdoctoral Fellow	David J. VanderWeele, MD, PhD Kathleen Kelly, PhD	National Cancer Institute Laboratory of Genitourinary Cancer Pathogenesis

Professional Positions

2021-	Research Assistant Professor	Jacobs School of Medicine and Biomedical Sciences, University at Buffalo
2015-2017	Graduate Research Assistant	Indiana University School of Medicine
2010-2012	Laboratory Technician	Roswell Park Cancer Institute
2007-2009	Undergraduate Research Assistant	State University of New York at Fredonia

Committee Service

2021-	University at Buffalo Cancer Research Consortium Seminar Series, Co-leader 2021-Present	University at Buffalo
2017-2018	Center for Cancer Research Fellows & Young Investigators (CCR-FYI), Association, CCR/NCI/NIH	National Institutes of Health

Professional Societies

2019-	Prostate Cancer Foundation	
	2020-	Leader, Androgen Receptor Working Group
2017-	Cancer Epigenetic Society (Member ID: 480)	
	2017-	Assistant Member
2011-	American Association for Cancer Research	
	2011-2012	Affiliate Member
	2012-	Associate Member
2012 -	American Society of Clinical Oncology	
	2012-2020	Member
	2021 -	Active Member

Grant Review Activities

2020-	Young Investigator Award	Prostate Cancer Foundation
	2020, 2021	Reviewer

Editorial Activities

Ad-hoc Reviewer	Journal	Number of manuscripts
	Urology	1
	Molecular Cancer Therapeutics	1

Other Editorial Roles

2021-	Review Editor	Frontiers in Oncology Frontiers Cell and Developmental Biology

Honors and Prizes

2021	CTSI Scholar Award	University at Buffalo Clinical and Translational Science Institute	Career Development Award
2019	Young Investigator Award	Prostate Cancer Foundation	Career Development Award
2018	NIH Summer Research Mentor Award	National Institutes of Health	Mentor award
2016	Scholar-in-Training Award	Indiana University, School of Medicine	Travel award
2015	Cancer Research Day 2nd Prize	Indiana University	Poster competition
2014	Genitourinary Research Retreat. 1st Prize	Roswell Park Cancer Institute	Poster competition
2009	Beta Beta Beta Biological Sciences honors	State University of New York at Fredonia Chapter.	Merit Honor
2007	President's International Student Scholar Award	State University of New York at Fredonia	Scholarship

Funded Projects

Current

2021- Targeting drivers of non-canonical AR cistrome associated with therapeutic resistance in advanced prostate cancer
Kaleida Health Foundation Troup Fund (PI)
We are using single cell transcriptomic sequencing and single cell ATAC-sequencing to identify enrichments of non-canonical AR cistrome in human cells lines and organoids that are resistant to enzalutamide and their drivers. We are also using high-through-put drug screen to determine their vulnerabilities which can be translated into the clinic

2019- Dynamics of Epigenetic Mechanisms in the Akt Signaling Pathway and Its Influences on Drug Response in Advanced Prostate Cancer
W81XWH 19 1 0715, DOD-CDMRP-PCRP Early Investigator (PI)
The major goal of this study is to identify the key role of epigenetic programming that links PI3K/AKT signaling and response to Androgen Receptor (AR) blockade, and to use well-characterized preclinical 3D organoid and patient derived xenograft (PDX) models to develop a more effective therapeutic strategy to target these pathways.

2019- Investigating Alterations in AR regulome following response to Kinase Inhibitors
19YOUN01, Prostate Cancer Foundation Young Investigator (PI)

We use high depth RNA/ChIP next generation sequencing and functional genomics to determine the molecular mechanism linking changes in AR regulome with kinase inhibition. The goal is to provide more informed optimal therapeutic strategies and secondly, our findings well as provide biomarkers for early detection of lethal PCa

Local Teaching and Training

2021	MED 400: Exploring Medical Research
	Basic Research I & II
2021	James Crawford, Summer College Student
2019	Anna Savan, Summer College Student

National and International Invited Teaching and Presentations

National

2021 Cancer Research Consortium
University at Buffalo, NY

2020 Targeting the PI3K/AKT pathway overcomes enzalutamide resistance by inhibiting induction of the glucocorticoid receptor (Selected poster, oral presentation)
Thirteenth Annual Multi-Institutional Prostate Cancer Program Retreat, Fort Lauderdale, FL

2016 EZH2 modulates sunitinibresistance by kinome reprogramming in renal cell carcinoma
Purdue University/ Indiana University Basic Urologic Research (IBUR) Symposium, IN

2016 Androgen receptor expression is associated with sunitinib resistance inrenal cell carcinoma models
Indiana University, School of Medicine, Indianapolis, IN

2015 Dissecting Mechanisms of Resistance to Anti-VEGF Therapies in Renal CellCarcinoma.
Roswell Park Cancer Institute, Buffalo, NY

2014 Nutrigenomics and Epigenetics in Genitourinary Cancer Therapy
RoswellPark Cancer Institute, Buffalo, NY

International

2018 Epigenetic alterations associated with resistance to therapy: Focus inadvance renal cell carcinoma
Imperial College London UK

Peer-Reviewed Abstracts

1. **Adelaiye, R.,** Leone, P., Paladino, J. and Lee. T. Microbial Source Tracking: Identifying Sources if Escherichia coli Pollution in Lake Erie. Poster presentation at 17th Annual Statewide CSTEP Student Conference and SeniorCreativity Exposition.

2. Masood, A., Miles K.M., Khan, N.H., **Adelaiye, R**., Akhtar, D., Ser, T., Chitta, K. and Chanan-Khan, A. An Investigational Proteasome Inhibitor MLN9708 (MLN2238) Induces Apoptosis in Human Multiple Myeloma Cells in vitro. Am. Soc. of Hematology.

3. Masood, A., Khan, N.H., Miles, K.M; Akhtar, D., **Adelaiye, R**., Advani, P., Sher, T., Chitta, K. and Chanan- Khan, A. Preclinical Evaluation of the proteasome inhibitor MLN9708 (MLN2238) in Chronic Lymphocytic Leukemia Cells. Am. Soc. of Hematology.

4. **R. Adelaiye**, L. Ellis, R. Pili. (2012) Targeting heat shock proteins: Novel strategies for treating prostate cancer.Proc AACR # 2766.

5. **R. Adelaiye**, E. Ciamporcero, KM Miles, P. Sotomayor, R. Pili. (2013). Sunitinib Dose- Escalation Overcomes Transient Drug Resistance in Clear Cell Renal Cell Carcinoma. Proc Anti-Angiogenesis #

6. E. Ciamporcero, KM. Miles, **R. Adelaiye**, S. Pizzimenti, G. Barrera, R. Pili (2013) Combination of Axitinib andcrizotinib in renal cell carcinoma models. Proc AACR #1618.

7. R. Pili, **R. Adelaiye**, KM Miles, E. Ciamporcero, P. Sotomayor, G. Bjarnason. (2013) Overcoming sunitinib- induced resistance by does escalation in renal cell carcinoma: evidence in animal models and patients. Proc ASCO#4582.

8. **R. Adelaiye**, KM Miles, E. Ciamporcero, H. Nguyen, R. Vessella, L. Fontana, R. Pili. (2013) Tumor growth inhibition and epigenetic changes following protein diet restriction in a human prostate cancer model. Proc AACR#4859.

9. **R. Adelaiye**, KM Miles, E. Ciamporcero, D. Conroy, S. Ramakrishnan, A. Orillion, S. Ku, M. Elbanna, L. Shen,S. Chintala, R. Pili. (2014) Epigenetic changes associated with resistance to sunitinib in human clear cell renalcell carcinoma models. Proc AACR #1375

10. A.Orillion, KM. Miles, L Shen, **R. Adelaiye**, E. Ciamporcero, S. Ramakrishnan, S. Ku, M. Elbanna, S. Chintala, R. Pili. (2014) Angiopoietin ½ inhibition impairs tumor growth in a orthotopic model of renal cell carcinoma.Proc AACR #1013

11. S. Ramakrishnan, S. Ku, W. Wetzig, D. Conroy, L. Shen, S. Chintala, P. Sotomayor, KM Miles, **R. Adelaiye**, E.Ciamporcero, A. Orillion, L. Ellis, G. Das, R. Pili. (2014) Evidence for hdac6 and er-α association in a subset ofclear cell renal cell carcinoma. Proc AACR #4061

12. S. Chintala, KM Miles, **R. Adelaiye**, R. Pili. (2014) Modulation of circulating microRNA's in serum of clear cellrenal cell carcinoma (ccRCC) patients treated with vorinostat and bevacizumab. Proc AACR #4395. Cancer Research, 74(19 Supplement), 4395-4395.

13. **R. Adelaiye**, S. Chintala, L. Shen, A. Orillion, E. Ciamporcero, M. Elbanna, KM Miles, B. Gillard, M.

Buck, R.Pili (2015). Inhibition of EZH2 Overcomes Resistance to Sunitinib in Clear Cell Renal Cell Carcinoma Models.Proc AACR. Cancer Research 75.15 Supplement (2015): 3508-3508.

14. **R. Adelaiye**, L. Shen, S. Chintala, A. Orillion, E. Ciamporcero, M. Elbanna, SK. Ku, KM Miles, B. Gillard, M. Buck, R. Pili (2015). Anti-Tumor and Anti-Metastatic Effect of Sunitinib in a Patient Derived Metastatic Clear Cell Renal Cell Carcinoma Xenograft Model. Proc AACR. Cancer Research 75.15 Supplement (2015): 4132- 4132.

15. **Remi Adelaiye-Ogala**, Sreenivasulu Chintala, Ashley, Piergiorgio Pettazzoni, May Elbanna, Ben Elzey, Kiersten Marie Miles, Chinghai Kao, Giulio F. Draetta, and Roberto Pili (2016). Androgen receptor expression is associated with sunitinib resistance in renal cell carcinoma models. Proc AACR (2016).

16. Ashley Orillion, **Remi Adelaiye-Ogala**, Li Shen, Eric Ciamporcero, Kiersten Marie Miles, May Elbanna, Sreenivasulu Chintala, Sreevani Arisa, Ben Elzey, Chinghai Kao, Luigi Fontana, Roberto Pili (2016). Methioninerestriction alters functional polarization of macrophages in a murine model of prostate cancer. Proc AACR (2016)

17. Ashley R. Orillion, Li Shen, **Remi Adelaiye-Ogala**, May Elbanna, Sreenivasulu Chintala, Sreevani Arisa, Roberto Pili (2016). The selective class I HDAC inhibitor Entinostat enhances the antitumor effect of PD-1 inhibition in a syngeneic orthotopic murine model of renal cell carcinoma. Proc AACR (2016)

18. May F. Elbanna, Eric Ciamporcero, **Remi Adelaiye**, Ashley Orillion, Sreenivasulu Chintala, Roberto Pili. (2016).Differential response to a dual PI3K/mTOR inhibitor in PIK3CA mutant urothelial cancer patient derived xenografts. Proc AACR (2016)

19. Sreenivasulu Chintala, **Remi Adelaiye-Ogala**, Ashley Orillion, Sreevani Arisa, May Elbanna, Roberto Pili (2016). Inhibition of SEC24D decreases exosome release of the tumor suppressor miR-605 in renal cellcarcinoma. Proc AACR (2016)

20. Nur P. Damayanti, Sreenivasulu Chintala, Ashley Orillion, **Remi Adelaiye-Ogala**, May F. Elbanna, Pete Hollenhorst and Roberto Pili. Delineating translocation renal cell carcinoma oncogenesis in cells harboring TFE3fusion with spliceosome machinery associated genes. Cancer Res July 1 2017 (77) (13 Supplement) 4475; DOI:10.1158/1538-7445.AM2017-4475.

21. Orillion AR, Chintala S, **Adelaiye-Ogala R**, Shen L, Damayanti N, Elbanna M, Arisa S, Elzey B, Kao C, FontanaL, Pili R. Abstract 250: Methionine restriction increases macrophage tumoricidal activity and significantly inhibits prostate cancer growth. Cancer Research. 2017;77(13 Supplement):250-. doi:10.1158/1538- 7445.Am2017-250.

22. Chintala S, **Adelaiye-Ogala R**, Orillion A, Arisa S, Elbanna M, Damayanti NP, Pili R. Abstract 94: Association of xCT overexpression with RTKI resistance and metastases in clear cell renal cell carcinoma. Cancer Research.2017;77(13 Supplement):94-. doi: 10.1158/1538-7445.Am2017-94.

23. **Adelaiye-Ogala RM**, Nguyen YT, Rodriguez-Nieves JA, Alilin AN, Agarwal S, VanderWeele D. Abstract 5787:Ipatasertib impairs tumor growth in androgen sensitive and castrate resistant prostate cancer: Evidence in preclinical models. Cancer Research. 2018;78(13 Supplement):5787-. doi:10.1158/1538-7445.Am2018-5787.

Peer-Reviewed Publications of Original Research

1. Ellis L, Lehet K, Ramakrishnan S, **Adelaiye R**, Miles KM, Wang D, Liu S, Atadja P, Carducci MA, Pili

R. Concurrent HDAC and mTORC1 inhibition attenuate androgen receptor and hypoxia signaling associated with alterations in microRNA expression. PLoSOne.2011;6(11):e27178. Epub 2011/11/17. doi:10.1371/journal.pone.0027178; PMCID: Pmc3210144.

2. Ellis L, Lehet K, Ramakrishnan S, **Adelaiye R**, Pili R. Development of a castrate resistant transplant tumor model of prostate cancer. Prostate. 2012;72(6):587-91. doi:10.1002/pros.21465. PubMed PMID: 21796655; PMCID: PMC3298731.

3. Fontana L#, **Adelaiye RM#,** Rastelli AL, Miles KM, Ciamporcero E, Longo VD, Nguyen H, Vessella R, Pili R.Dietary protein restriction inhibits tumor growth in human xenograft models. Oncotarget. 2013;4(12):2451-61. doi:10.18632/oncotarget.1586. PubMed PMID: 24353195; PMCID: PMC3926840.

4. Ku S, Lasorsa E, **Adelaiye R**, Ramakrishnan S, Ellis L, Pili R. Inhibition of Hsp90 augments docetaxel therapy in castrate resistant prostate cancer. PLoSOne. 2014;9(7):e103680. Epub 2014/07/30. doi: 10.1371/journal.pone.0103680; PMCID: Pmc4114978.

5. Shen L, Sundstedt A, Ciesielski MJ, Miles KM, Celander M, **Adelaiye R**, Orillion A, Ciamporcero E, Ramakrishnan S, Ellis L, Fenstermaker RA, Abrams SI, Eriksson H, Leanderson T, Olson A, Pili R. Tasquinimodmodulates suppressive myeloid cells and enhances cancer immunotherapies in murine models. Cancer ImmunolRes. 2014. Epub 2014/11/06. doi:10.1158/2326-6066.cir-14-0036.

6. Miles KM, Seshadri M, Ciamporcero E, **Adelaiye R**, Gillard B, Sotomayor P, Attwood K, Shen L, Conroy D, Kuhnert F, Lalani AS, Thurston G, Pili R. Dll4 blockade potentiates the anti-tumor effects of VEGF inhibition inrenal cell carcinoma patient-derived xenografts. PLoSOne. 2014;9(11):e112371. Epub 2014/11/14. doi: 10.1371/journal.pone.0112371; PMCID: Pmc4231048.

7. Gotink KJ, Broxterman HJ, Honeywell RJ, Dekker H, de Haas RR, Miles KM, **Adelaiye R**, Griffioen AW, Peters GJ, Pili R, Verheul HM. Acquired tumor cell resistance to sunitinib causes resistance in a HT-29 human colon cancer xenograft mouse model without affecting sunitinib biodistribution or the tumor microvasculature. Oncoscience. 2014;1(12):844-53. doi:10.18632/oncoscience.106. PubMed PMID: 25621299; PMCID: PMC4303892.

8. Ciamporcero E, Miles KM, **Adelaiye R**, Ramakrishnan S, Shen L, Ku SY, Pizzimenti S, Sennino B, Barrera G, Pili R. Combination strategy targeting VEGF and HGF/c-met in human renal cell carcinoma models. Mol CancerTher. 2014. Epub 2014/11/09. doi: 10.1158/1535- 7163.mct-14-0094.

9. **Adelaiye R**, Ciamporcero E, Miles KM, Sotomayor P, Bard J, Tsompana M, Conroy D, Shen L, Ramakrishnan S, Ku SY, Orillion A, Prey J, Fetterly G, Buck M, Chintala S, Bjarnason GA, Pili R. Sunitinib dose escalation overcomes transient resistance in clear cell renal cell carcinoma and is associated with epigenetic modifications. Mol Cancer Ther. 2015;14(2):513-22. doi:10.1158/1535-7163.MCT-14-0208. PubMed PMID: 25519701; PMCID: PMC4326587.

10. Pili R, Liu G, Chintala S, Verheul H, Rehman S, Attwood K, Lodge MA, Wahl R, Martin JI, Miles KM, PaesanteS, **Adelaiye R**, Godoy A, King S, Zwiebel J, Carducci MA. Combination of the histone deacetylase inhibitor vorinostat with bevacizumab in patients with clear-cell renal cell carcinoma: a multicentre, single-arm phase I/IIclinical trial. Br J Cancer. 2017;116(7):874- 83. Epub 2017/02/22. doi: 10.1038/bjc.2017.33. PubMed PMID: 28222071; PMCID: PMC5379145.

11. Wentink MQ, Verheul HMW, Pal SK, George S, Voortman J, Danchaivijitr P, **Adelaiye R**, Poslinski D, Groman A, Hutson A, Pili R. Phase I Study of Dalteparin in Combination with Sunitinib in Patients with Metastatic Clear Cell Renal Carcinoma. Clin Genitourin Cancer. 2017. Epub 2017/08/07. doi:

10.1016/j.clgc.2017.07.004. PubMed PMID: 28781191.

12. **Adelaiye-Ogala R**, Budka J, Damayanti NP, Arrington J, Ferris MW, Hsu CC, Chintala S, Orillion AR, Miles KM, Shen L, Elbanna M, Ciamporcero E, Arisa S, Pettazzoni P, Draetta GF, Seshadri M, Hancock BA, RadovichM, Kota J, Buck M, Keilhack H, McCarthy BP, Persohn SA, Territo PR, Zang Y, Irudayaraj J, Tao AW, Hollenhorst P, Pili R. EZH2 modifies sunitinib resistance in renal cell carcinoma by kinome reprogramming. Cancer Res. 2017. doi:10.1158/0008-5472. CAN-17-0899. PubMed PMID: 28978636.

13. Orillion A, Hashimoto A, Damayanti N, Shen L, **Adelaiye-Ogala R**, Arisa S, Chintala S, Ordentlich P, Kao C, Elzey B, Gabrilovich D, Pili R. Entinostat Neutralizes Myeloid-Derived Suppressor Cells and Enhances the Antitumor Effect of PD-1 Inhibition in Murine Models of Lung and Renal Cell Carcinoma. Clin Cancer Res. 2017;23(17):5187-201. Epub 2017/07/13. doi:10.1158/1078-0432.CCR-17-0741. PubMed PMID: 28698201; PMCID: PMC5723438.

14. **Adelaiye-Ogala R**, Damayanti NP, Orillion AR, Arisa S, Chintala S, Titus MA, Kao C, Pili R. Therapeutic Targeting of Sunitinib-Induced AR Phosphorylation in Renal Cell Carcinoma. Cancer Res. 2018;78(11):2886- 96. Epub 2018/03/25. doi:10.1158/0008-5472.CAN-17-3386. PubMed PMID: 29572225.

15. Damayanti NP, Budka JA, Khella HWZ, Ferris MW, Ku SY, Kauffman EC, Wood AC, Ahmed K, Chintala VN, **Adelaiye-Ogala R**, Elbanna M, Orillion AR, Chintala S, Kao C, Linehan WM, Yousef GM, Hollenhorst P, Pili R. Therapeutic targeting of TFE3/IRS-1/PI3K/mTOR axis in translocation renal cell carcinoma. Clin Cancer Res.2018. Epub 2018/08/01. doi:10.1158/1078- 0432.CCR-18-0269. PubMed PMID: 30061365.

16. Ashley Orillion, Nur P. Damayanti, Li Shen, **Remi Adelaiye-Ogala**, Hayley Affronti, May Elbanna, Sreenivasulu Chintala, Michael Ciesielski, Luigi Fontana, Chinghai Kao, Bennett D. Elzey, Timothy L. Ratliff, David E. Nelson, Dominic Smiraglia, Scott I. Abrams and Roberto Pili. Dietary Protein Restriction Reprograms Tumor- Associated Macrophages and Enhances Immunotherapy DOI: 10.1158/1078-0432.CCR-18-0980

17. Elbanna M, Orillion AR, Damayanti NP, **Adelaiye-Ogala R**, Shen L, Miles KM, Chintala S, Ciamporcero E, Ramakrishnan S, Ku SY, Rex K, Caenepeel S, Coxon A, Pili R. Dual inhibition of angiopoietin-TIE2 and MET alters the tumor microenvironment and prolongs survival in a metastatic model of renal cell carcinoma. Mol Cancer Ther. 2019 Oct 3. pii: molcanther.1202.2018. doi: 10.1158/1535-7163.MCT-18-1202

18. **Remi M. Adelaiye-Ogala**, Berkley Gryder, Yen Thi Minh Nguyen, Aian Neil Alilin, Adlai Grayson, Keith H. Jansson, Michael L. Beshiri, Supreet Agarwal, Jose Antonio Rodriguez-Nieves, Brian Capaldo, Kathleen Kelly and David J. VanderWeele. Targeting the PI3K/AKT pathway overcomes enzalutamide resistance by inhibitinginduction of the glucocorticoid receptor. Mol Cancer Ther. 2020 May 5. doi: 10.1158/1535-7163.MCT-19-0936

PhD Dissertation (Defended 10/2016)

Epigenetic alterations associated withresistance to anti-VEGR kinase inhibitors in advanced clear cell renal cell carcinoma

Published OnlineFirst May 5, 2020; DOI: 10.1158/1535-7163.MCT-19-0936

Targeting the PI3K/AKT Pathway Overcomes Enzalutamide Resistance by Inhibiting Induction of the Glucocorticoid Receptor

Remi Adelaiye-Ogala[1], Berkley E. Gryder[2], Yen Thi Minh Nguyen[1], Aian Neil Alilin[1], Adlai R. Grayson[1], Wardah Bajwa[1], Keith H. Jansson[1], Michael L. Beshiri[1], Supreet Agarwal[1], Jose Antonio Rodriguez-Nieves[1], Brian Capaldo[1], Kathleen Kelly[1], and David J. VanderWeele[1,3]

ABSTRACT

The PI3K–AKT pathway has pleiotropic effects and its inhibition has long been of interest in the management of prostate cancer, where a compensatory increase in PI3K signaling has been reported following androgen receptor (AR) blockade. Prostate cancer cells can also bypass AR blockade through induction of other hormone receptors, in particular the glucocorticoid receptor (GR). Here we demonstrate that AKT inhibition significantly decreases cell proliferation through both cytostatic and cytotoxic effects. The cytotoxic effect is enhanced by AR inhibition and is most pronounced in models that induce compensatory GR expression. AKT inhibition increases canonical AR activity and remodels the chromatin landscape, decreasing enhancer interaction at the GR gene (*NR3C1*) locus. Importantly, it blocks induction of GR expression and activity following AR blockade. This is confirmed in multiple *in vivo* models, where AKT inhibition of established xenografts leads to increased canonical AR activity, decreased GR expression, and marked antitumor activity. Overall, our results demonstrate that inhibition of the PI3K/AKT pathway can block GR activity and overcome GR-mediated resistance to AR-targeted therapy. Ipatasertib is currently in clinical development, and GR induction may be a biomarker to identify responsive patients or a responsive disease state.

Introduction

Suppression of androgen synthesis and androgen receptor (AR) activity via chemical castration and/or an AR antagonist is the mainstay of systemic prostate cancer therapy (1). While almost all patients have an initial favorable response, inevitably resistance develops, and patients relapse with progressive disease (2, 3). Multiple mechanisms can promote resistance, including reactivation of AR through amplification of the AR gene and/or an enhancer, mutation, or expression of AR variants (4–7). Expression of alternate hormone receptors with overlapping downstream targets can also promote resistance. Recent preclinical and clinical studies suggest the glucocorticoid receptor (GR) is the primary hormone receptor whose activity confers resistance to AR-targeted therapy (8, 9). Resistance to AR-targeted therapy through induction of GR expression is the basis for multiple clinical trials combining inhibition of GR with enzalutamide (NCT03674814, NCT02012296, and NCT03437941).

There is also cross-talk between androgen signaling and PI3K/AKT pathway activation. The PI3K/AKT pathway is a complex, branching and looping signaling pathway that is involved in survival, proliferation, metabolism, and growth pathways (10). In prostate cancer, castration or AR antagonism has been shown to increase phosphorylation of AKT and downstream targets, and loss of PTEN, a negative regulator of the PI3K/AKT pathway, correlates with decreased AR activity (11). Ipatasertib is a potent, pan-AKT inhibitor that binds AKT in an ATP-competitive manner, consequently disrupting its effect on downstream targets (12, 13). It has activity in combination with the CYP17A1 inhibitor abiraterone plus prednisone in metastatic castrate-resistant prostate cancer (mCRPC; ref. 14). Given encouraging preliminary data, there are several ongoing clinical trials assessing the therapeutic benefit of ipatasertib in combination with other antineoplastic agents, including in prostate cancer (15–17); however, the optimal setting for AKT inhibition is unknown.

Here we demonstrate that inhibition of AKT has a cytostatic effect on prostate cancer cells despite increasing AR activity and is cytotoxic in combination with AR antagonism. Moreover, among the effects of PI3K/AKT pathway inhibitors, they block the induction of GR seen in response to AR blockade, which is associated with remodeling of the chromatin landscape and effects on transcriptional regulation. In *in vivo* models of established tumors, AKT inhibition enhances canonical AR activity, blocks GR expression, and demonstrates marked antitumor activity.

Materials and Methods

Cell lines, patient-derived xenografts, and reagents

LNCaP and C4-2 were purchased from ATCC (ATCC.com). LAPC4 and LREX were provided by Dr. Sawyers, Memorial Sloan Kettering Cancer Center. LNCaP, C4-2, 22Rv1, and LREX cells were cultured in RPMI1640+GlutaMAX (Gibco, Life Technologies), 10% FBS (Gibco, Life Technologies), and 1% penicillin/streptomycin (Corning). LAPC4 cells were cultured in Iscove modified Dulbecco medium supplemented with 10% FBS and 1% penicillin/streptomycin. For androgen deprivation *in vitro* studies, 10% CSS (Gibco, Life Technologies) was used in place of 10% FBS. All cell lines were tested for *Mycoplasma* contamination (PCR Mycoplasma Detection; primer

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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sequence in Supplementary Table S1) every 6 months. LuCaP patient-derived xenograft (PDX) models (provided by Dr. E. Corey and Dr. R.L. Vessella, The University of Washington; Seattle, WA) were grown in organoid culture in advanced DMEM/F12 media with supplements, as published previously (18). For *in vitro* studies, ipatasertib (Chemietek) and enzalutamide (Selleckchem) were dissolved in DMSO. CellTitre Glo Assay (Promega) was used to assess cell viability. For *in vivo* studies, ipatasertib [Division of Cancer Treatment and Diagnosis (DCTD), NCI Developmental Therapeutics Program (DTP)] and enzalutamide (DCTD, NCI DTP) were dissolved in 1:1 of labrasol (Gattefosse) to PEG400 (Sigma).

Western blot analysis

Cell lines and xenograft tumors collected at the end of treatment (EOT) time point were lysed using standard protocol. Lysates generated were used to perform immunoblot as described previously (19). Primary antibodies are diluted 1:1,000 and are from Santa Cruz Biotechnology (AR) or Cell Signaling Technology [AKT, p-AKT(Thr308), p-AKT (Ser483), GR, P70S6K, p-P70S6K, 4EBP1, p-4EBP1, pS6r, KLK3, NKX3.1, H3, and GAPDH]. Secondary antibodies against rabbit (1:5,000; EMD Millipore) or mouse (1:5,000; EMD Millipore) were used followed by band detection using Amersham ECL Prime Western blotting detection reagent (GE Healthcare Lifesciences) as per manufacturer's instructions and visualized using ChemiDoc Touch Imaging system (Bio-Rad). Quantitative measurements of Western blot analysis were performed with ImageJ and GraphPad Prism8 software.

qRT-PCR analysis

mRNA extracts from cell lines and xenograft collected post EOT time points were used to perform qRT-PCR as described previously (19) for detection of *KLK3*, *NKX3-1*, and *NR3C1* (Supplementary Table S1). PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems). PCR arrays were used to evaluate expression of a panel of 84 genes putatively regulated by GR (PAHS-154ZA) or by AR (PAHS-142ZA; Qiagen). For the AR array, seven genes were removed from further analysis because their expression was increased by enzalutamide.

RNA-Seq analysis

RNA was extracted, quantified, and profiled for quality using a Bioanalyzer (Agilent). Poly-A–enriched and Illumina–barcoded libraries were prepared and sequenced on a NextSeq 500 (Illumina), to a depth of 30 million base pairs (150 bp paired end) at the Illumina Sequencing Facility, Center for Cancer Research, NIH Frederick Campus. Paired-end RNA sequencing (RNA-seq) reads were mapped to UCSC reference genome for hg19 with STAR (https://github.com/alexdobin/STAR), then transcripts per million (TPM) was calculated for each gene using RNA-Seq by Expectation Maximization (RSEM) (https://deweylab.github.io/RSEM). Gene set enrichments analysis (GSEA) was performed using preranked gene lists of $\log_2$ fold change comparison of TPM values between each treatment and the DMSO control. GSEA data visualization was performed using custom R scripts (available here: https://github.com/GryderArt/VisualizeRNAseq/).

ChIP-seq analysis

Chromatin was isolated from cells using ChIP-IT High Sensitivity Kit (Active Motif) according to the manufacturer's protocol. Sheared chromatin was incubated with antibody against H3K27ac (Active Motif). Protein–DNA bound complex was immunoprecipitated, followed by reverse cross-linking and DNA purification. ChIP-seq samples were pooled and sequenced on NextSeq (Illumina) using Accel-NGS 2S Plus DNA Library Kit (low input; Swift) and single-end sequencing. All the samples have percent of Q30 bases above 92%. All the samples have yields between 15 and 57 million pass filter reads. Samples were trimmed for adapters using trimmomatic software before the alignment. Single-end reads were mapped to hg19 with Burrows–Wheeler Aligner, Tile data file (TDF) were made for visualization in Integrative Genomics Viewer (IGV) using igvtools count (https://software.broadinstitute.org/software/igv/igvtools_commandline). MACS2 was used to perform peak calling with a threshold of 1E-7. HOMER was used to identify motif enrichment (http://homer.ucsd.edu) at sites of H3K27ac, and *P* values were visualized in GraphPad Prism. Chromatin folding was inferred from analysis of public HiC data (20; https://aidenlab.org/juicebox/).

Gene overexpression and knockdown assays

For GR (*NR3C1*) overexpression transfections, cells were transfected with 1–2 μg *NR3C1* (Myc- DDK-tagged) expressing cDNA (OriGene Technologies, Inc.) using TurboFectin 8.0 transfection reagent according to manufacturer's protocol (OriGene Technologies, Inc.) in LREX and LNCaP cells. For gene silencing of *AR* and *NR3C1* gene expressions, LREX cells were transduced with lentivirus particles of four different sequences per target gene or a nonsilencing sequence as control (OriGene Technologies, Inc.) according to the manufacturer's guidelines.

In vitro assays

Two-dimensional *in vitro* experiments

LNCaP and LAPC4 cells were plated in 24-well plates. Following overnight incubation, cells received fresh media containing 10% CSS and 1% penicillin/streptomycin. Cells were treated with either 10 nmol/L R1881 (Sigma), 100 nmol/L ipatasertib or combination of R1881 and ipatasertib. LREX, 22Rv1, and C4-2 cells were prepared as stated previously and treated with either 100 nmol/L ipatasertib, 2 μmol/L enzalutamide or combination of both ipatasertib and enzalutamide. Viable cells were determined using CellTitre Glo assay 24 to 168 hours posttreatment in accordance with manufacturer's instructions and absorbance read using NanoQuant Infinite M200 Pro reader (TECAN). In parallel, cells were plated in 10 cm dishes and collected 48 hours after treatment for RNA and protein analysis.

In vivo studies

Xenograft models

LuCaP 136 and LuCaP 147 are well-characterized prostate cancer PDX models (21) established at The University of Washington (Seattle, WA). LuCaP 136CR-N is a castrate-resistant prostate cancer developed from the parental LuCaP 136 model that has undergone several passages in castrate mice to select for castrate-resistant tumors. All LuCaPs were validated using short tandem repeat analysis.

Tumor implantation

All preclinical *in vivo* experiments were performed in accordance with an NCI Animal Care and Use Committee approved protocol. Six-week-old Athymic Nude-*Foxn1*^{nu} male mice were housed in a sterile, pathogen-free facility and maintained in a temperature- controlled room under a 12-hour light/dark schedule with water and food ad libitum. All mice were operated under sedation with oxygen and isoflurane. Ibuprofen and/or buprenorphine was administered post-surgery. Androgen-sensitive prostate cancer xenograft (PDX) models LuCaP 136 or 147 were implanted subcutaneously under the left flank of intact mice. When tumors were established and reached an average

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volume of approximately 500 mm^3, mice were randomized and placed in either control group or treatment groups ($n = 8$/group for LuCaP 136; $n = 6$/group for LuCaP 147). Mice received ipatasertib treatment (100 mg/kg, 5 days on 2 days off; oral gavage), androgen deprivation therapy (surgical castration) or a combination of both. For CRPC models, LuCaP 136CR-N or LREX cells were implanted subcutaneously under the left flank of castrated mice. When tumors were established and reached approximately 500 mm^3, mice were randomized and placed in either control group or treatment groups ($n = 8$/group). Mice received ipatasertib treatment (100 mg/kg, 5 days on 2 days off; oral gavage), enzalutamide (10 mg/kg, 5 days on 2 days off; oral gavage) or a combination of both. Tumor burden was assessed twice per week by caliper measurement of two diameters of the tumor ($L \times W = mm^2$) and reported as tumor volume [$(L \times W^2)/2 = mm^3$]. Body weights were assessed using a weighing scale and recorded in grams. Tumor tissues were excised, weighed, and snap frozen for further analysis.

Statistical analysis

Statistical analysis was performed using GraphPad prism (version 7.0a). In brief, data analyses are expressed as the mean + SEM unless otherwise stated. Statistical significance where appropriate was evaluated using a two-tailed Student *t* test when comparing two groups, or by one-way ANOVA, using the student–Newman–Keuls posttest for multiple comparisons. A *P* value, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, was considered significant; ns, not significant, or otherwise stated.

Results

The pan-AKT inhibitor ipatasertib decreases cell viability in an enzalutamide-resistant prostate cancer cell line

The PI3K/AKT signaling pathway is hyperactive in 50%–60% of advanced PCa (22), and the AKT inhibitor ipatasertib is currently in clinical development. We sought to determine the effects of ipatasertib (100 nmol/L) or enzalutamide in models representing CRPC. We utilized 22RV1, which is known to harbor full-length AR and ARV7, C4-2 (LNCAP/AR-derived CRPC) and LREX (LNCAP/AR-resistant to Enzalutamide) models. Cells were exposed to enzalutamide (2 μmol/L) or ipatasertib (100 nmol/L) in the setting of androgen deprivation. Response to enzalutamide was modest in 22Rv1 and C4-2 cell lines, as was response to ipatasertib (**Fig. 1A**, first and second panel). In contrast, LREX, which is poised to upregulate GR expression to achieve enzalutamide resistance (8), was confirmed to be completely resistant to enzalutamide but had a marked response to ipatasertib (**Fig. 1A**, third panel). Consistent with its mechanism of action, the effect of ipatasertib on cell viability correlated with inhibition of AKT signaling, which is manifest as an increase in AKT phosphorylation (due to its ability to protect against phosphatases) and decrease in phosphorylation of downstream targets (**Fig. 1B**). As expected, enzalutamide decreased AR activity, demonstrated by decreased expression of canonical AR targets PSA and NKX3.1 in C4-2 and LREX cells, though not in 22RV1 cells, which express the AR variant AR-V7. Interestingly, C4-2 and LREX had increased expression of these canonical AR targets in the presence of ipatasertib. To determine whether ipatasertib has an anticancer effect in hormone-sensitive prostate cancer (HSPC), we tested its effect in the well-characterized HSPC cell lines LNCaP and LAPC4. Both models of HSPC were more sensitive to ipatasertib than to androgen withdrawal (Supplementary Fig. S1A). Western blot analysis confirmed ipatasertib inhibited AKT signaling (Supplementary Fig. S1B). A time course of ipatasertib in LNCAP and LREX cells confirmed early and sustained effects on AKT activity (Supplementary Fig. S1C). Taken together, these results indicate heterogenous response to ipatasertib, with significant antitumor effect in models of CRPC and HSPC. Among all three CRPC models tested, ipatasertib was most active in the enzalutamide-resistant CRPC (LREX) cells.

Ipatasertib induces cell-cycle arrest as monotherapy and apoptosis in combination with enzalutamide

The LREX cell line was derived from LNCaP/AR with *in vivo* selection for enzalutamide resistance (8, 23). We evaluated response to enzalutamide and ipatasertib alone and in combination in a dose-dependent manner for 48 hours. We confirmed that LREX cells were resistant to enzalutamide at 10 μmol/L, with no difference in cell viability compared with DMSO-treated cells (**Fig. 2A**). However, we observed response to ipatasertib single agent in a dose-dependent manner which was enhanced when combined with enzalutamide (**Fig. 2A**). Similarly, in a time-dependent manner (24–168 hours of treatment) using single dose ipatasertib (100 nmol/L) and enzalutamide, we again observed no response to enzalutamide (**Fig. 2B**). LREX cells continue to be sensitive to ipatasertib over time, with approximately 50% growth suppression. Combination of ipatasertib and enzalutamide led to almost complete growth suppression (**Fig. 2B**).

To determine the mode of cell death following ipatasertib alone or when combined with enzalutamide, we performed cell-cycle analysis using propidium iodide (PI) after 24 hours of treatment. Single agent ipatasertib induced a G_0–G_1 cell-cycle arrest (**Fig. 2C**), and when combined with enzalutamide we found an increased subG1 population, indicative of apoptosis.

We confirmed the apoptotic cell death using annexin V/PI staining of live cells (**Fig. 2D**). Indeed, we observed increased apoptosis in cells treated with both ipatasertib and enzalutamide compared with single agents. Taken together, our data suggest that ipatasertib halts cell growth primarily by inducing cell-cycle arrest, and when combined with enzalutamide, it induces apoptotic cell death.

Inhibition of AKT blocks the induction of GR expression and activity through chromatin landscape reorganization

Induction of compensatory GR expression has been reported in a subset of enzalutamide-resistant patients with CRPC as a mechanism of resistance to AR blockade (8). Given the role of GR in enzalutamide resistance in LREX, we sought to determine the effect of AKT inhibition on GR. We examined expression of the GR protein in LREX cells by Western blot analysis following AKT and AR inhibition for 2 days. LREX cells in androgen-deprived media induced GR protein expression, which ipatasertib completely inhibited (**Fig. 3A**, top). LREX exposed to enzalutamide induced even higher expression of GR, which was significantly decreased when also exposed to ipatasertib (**Fig. 3A**, top). LNCaP have also been shown to induce GR expression, though over longer-term androgen inhibition (24). In LNCaP cells, we observed induction of GR expression after 7 days of exposure to enzalutamide, which is completely blocked by exposure to ipatasertib (Supplementary Fig. S2A).

To determine whether the decrease in GR expression correlates with GR activity, LREX cells were transfected with a luciferase reporter whose expression is driven by multiple copies of the GR response element. When these cells were exposed to 100 nmol/L ipatasertib for 48 hours, luciferase production decreased significantly. Conversely, when these cells were exposed to enzalutamide, they had increased luciferase production (**Fig. 3A**, middle). To evaluate GR activity in a more physiologic setting, we performed a PCR array using 84 putative GR-regulated genes. Confirming the luciferase assay finding,

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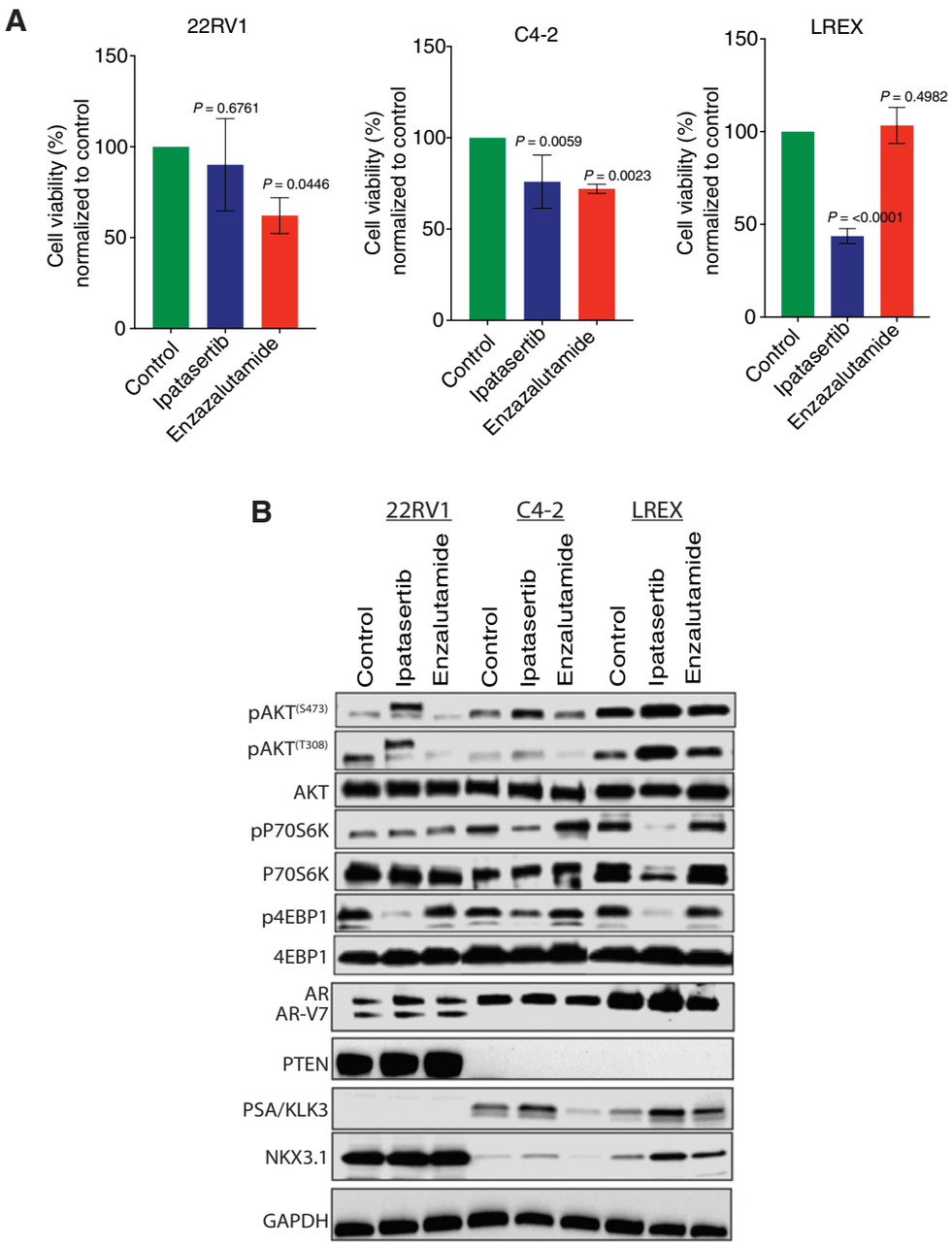

Figure 1.

Ipatasertib as single agent is highly potent in enzalutamide-resistant CRPC. **A,** Assessment of ipatasertib as single agent across prostate cancer modeling castration resistance (22RV1 and C4-2), show marginal response to the pan-AKT inhibitor ipatasertib. LREX, which induces GR to achieve enzalutamide resistance, shows robust response to ipatasertib. **B,** Western blot analysis indicates inhibitory effects on AKT phosphorylation and downstream targets. Immunoblot assay also indicates PTEN status, AR, AR-V7, and AR downstream targets following exposure to treatment. Graphs are presented as mean ± SD.

ipatasertib lead to decreased expression of a broad range of GR-regulated genes, indicating decreased GR activity (**Fig. 3A**, bottom).

LREX cells have a dynamic reprogramming system with enzalutamide, as these cells are poised to switch on GR for survival when faced with AR blockade. This dynamic on and off switch suggests that there is an epigenetic influence on gene expression modulated via typical enhancers or superenhancers. Epigenetic processes such as histone modifications at *cis*-regulatory elements can affect gene transcription independent of their orientation or distance via enhancers (25). H3K27ac has been established as an important mark of enhancers which distinguishes between active and inactive regions (active referring to a positive influence on the expression of proximal genes; ref. 26), hence, regions with deposits of H3K27ac are often associated with enhanced gene activity (26, 27). We performed chromatin

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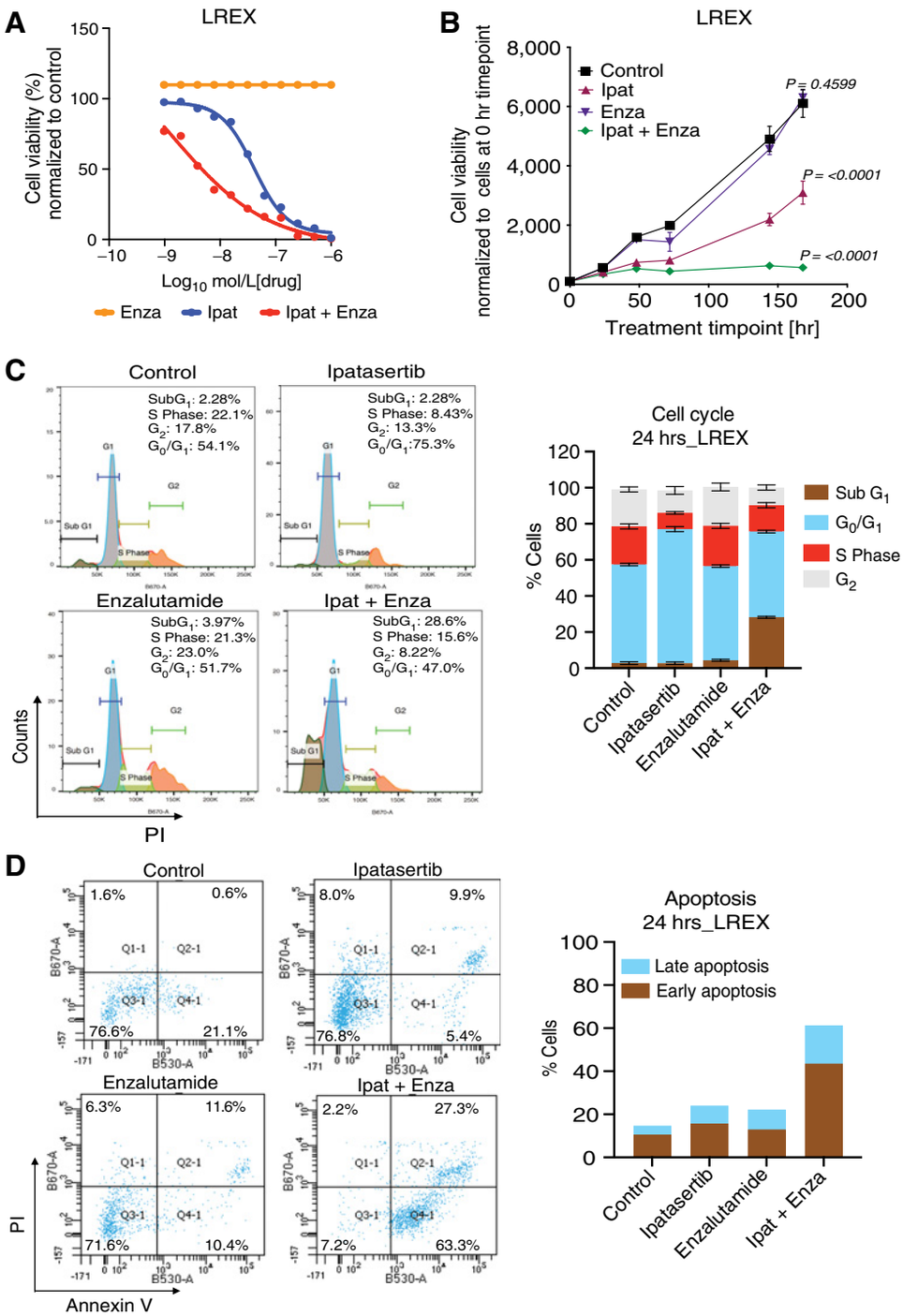

Figure 2.

Ipatasertib overrides enzalutamide resistance, inducing cell cycle G_0–G_1 arrest and apoptosis. **A,** Cell proliferation assay with increasing concentrations of ipatasertib, single dose enzalutamide (2 μmol/L) or combination of both drugs shows the shift in cells treated with both ipatasertib and enzalutamide compared with ipatasertib alone. **B,** Time-dependent cell viability indicates that LREX cells in CSS are resistant to enzalutamide, which is overcome by adding ipatasertib. **C,** Flow cytometry analysis of cell cycle revealed that ipatasertib alone induces cell-cycle arrest at the G_0–G_1 phase and there is an increased sub-G_1 population when combined with enzalutamide. **D,** Apoptosis analysis using annexin V/PI dual-stain assay indicates increased apoptosis in cells treated with both ipatasertib and enzalutamide.

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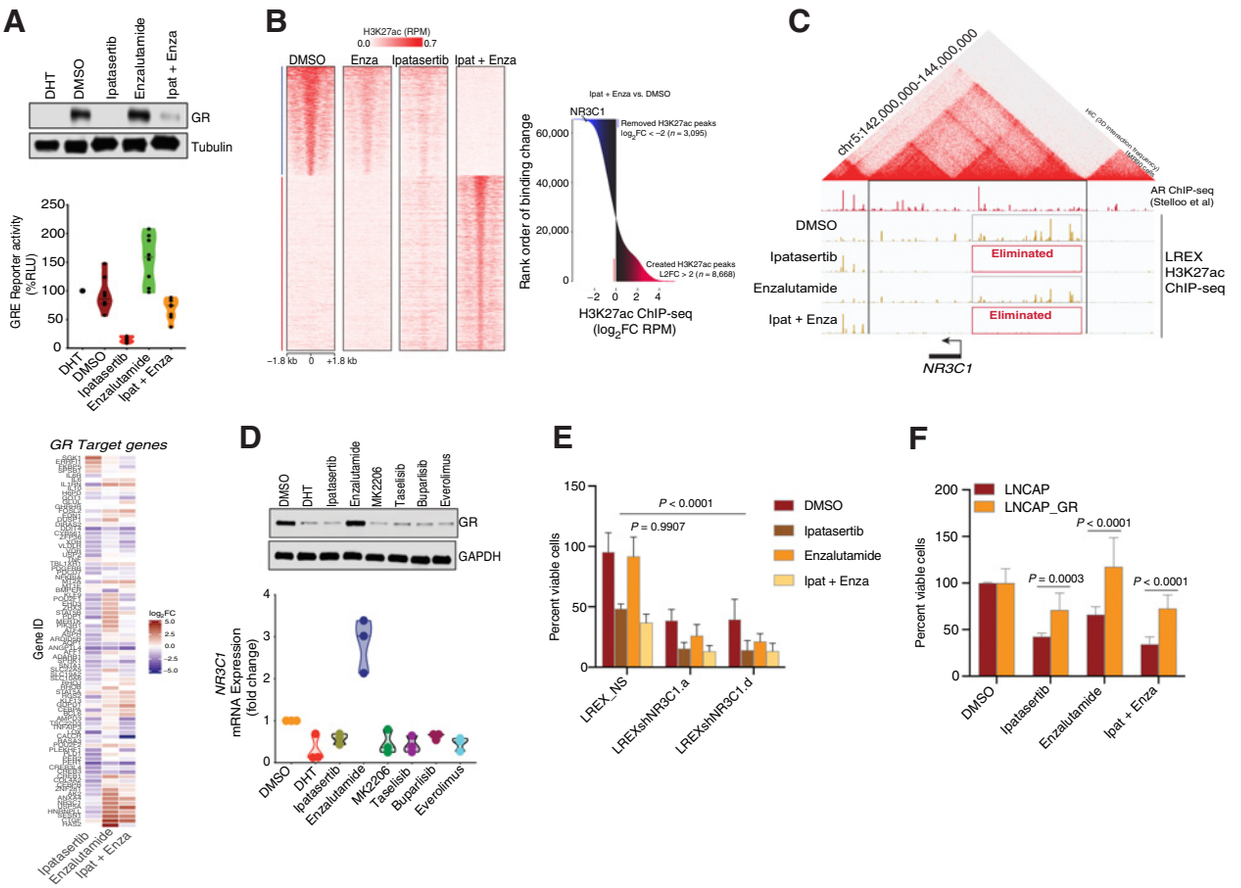

Figure 3.

AKT inhibition blocks induction of GR expression and activity. **A,** Western blot analysis indicates that GR expression is induced in LREX cells in the setting of 2 days of androgen deprivation or enzalutamide and inhibited by the pan-AKT inhibitor ipatasertib (top). Luciferase production from a reporter driven by tandem GRE elements in LREX cells demonstrates decreased GR activity with ipatasertib and increased GR activity with enzalutamide (middle). Grid heatmap of PCR array evaluating putative GR target genes indicating decreased expression of GR-regulated genes with ipatasertib and increased expression after AR inhibition with enzalutamide (bottom). **B,** H3K27ac ChIP signal heatmap across treatment groups (right). Ranking order of change in H3k27 acetylation upon treatment with ipatasertib and enzalutamide compared with DMSO (left). **C,** HiC three-dimensional interaction frequency pyramid and H3K27ac signal tracks (orange) shows regions upstream of the GR gene NR3C1 have absence of enhancer-interaction with ipatasertib treatment or combination treatment. **D,** Western blot (top) and qRT-PCR analyses (bottom) demonstrate that induction of GR protein or NR3C1 mRNA in the absence of androgen in LREX cells is blocked by synthetic androgen (R1881), AKT inhibitors ipatasertib or MK-2206, PI3K inhibitors taselisib (GDC-0032) or buparlisib (BKM120), or mTOR inhibitor everolimus, but not enzalutamide. **E,** Cell viability assay using LREX cells with approximately 80% knockown GR gene (*NR3C1*) indicates resensitization of LREX cells to enzalutamide following *NR3C1* knockdown. **F,** Cell viability assay indicates decreased response to ipatasertib in LNCaP cells overexpressing GR compared with control cells. Graphs are presented as mean ± SD.

immunoprecipitation (ChIP) experiments for H3K27ac in the presence of DMSO, ipatasertib, enzalutamide, or the combination of both ipatasertib and enzalutamide, with RNA-seq performed in parallel. First, we looked at global changes of H3K27ac binding across all treatment groups and observed a gradual switch in H3K27ac bound regions in ipatasertib treatment compared with control or enzalutamide and a complete flip in cells treated with ipatasertib and enzalutamide (**Fig. 3B**, left). When we ranked genes in order of binding change, the GR gene, *NR3C1,* ranked among the top genes to have decreased H3K27ac deposits (**Fig. 3B**, right). To ensure we searched the same *cis*-regulatory space available to *NR3C1* in three-dimensions, we used published HiC data (28) identifying chromatin interactions inside the nucleus and found clear boundaries (black lines, **Fig. 3C**) of an insulated neighborhood in which active enhancers could directly influence *NR3C1* (29). Interestingly, regions on the *NR3C1* locus with enhancer deposition in the presence of enzalutamide or DMSO were completely eliminated with ipatasertib or combination of both ipatasertib and enzalutamide (**Fig. 3C**). The epigenetic shutdown of *NR3C1* expression was corroborated by RNA-seq and qRT-PCR (Supplementary Fig. S2B and S2C). Taken together, these data suggest AKT inhibition deactivates GR through *cis*-regulatory elements at the *NR3C1* locus that sense the depletion in PI3K–AKT signal.

To determine whether inhibition of GR expression is specific to inhibition of AKT by ipatasertib or occurs with antagonism of other PI3K/AKT pathway members, we exposed LREX cells to the AKT inhibitor MK2206, the PI3K inhibitors taselisib or buparlisib, or the mTOR inhibitor everolimus in the setting of androgen deprivation. Stimulation of AR with the synthetic androgen R1881 led to a decrease in GR expression. In contrast, AR inhibition with enzalutamide increased GR expression. Like androgen stimulation, all four inhibitors of the PI3K/AKT pathway decreased GR expression similar to that of ipatasertib (**Fig. 3D**). Western blot analysis confirmed that these

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effects occurred in the setting of PI3K/AKT pathway inhibition: PI3K inhibitors taselisib and buparlisib and the allosteric AKT inhibitor MK-2206 decreased phosphorylation of AKT and downstream targets, whereas the ATP-competitive inhibitor ipatasertib and the mTOR inhibitor everolimus decreased phosphorylation of downstream targets in the setting of increased AKT phosphorylation (Supplementary Fig. S3). Taken together, our results suggest that PI3K/AKT inhibitors remodel the chromatin landscape to block the induction of GR expression at the transcript level and resensitizes cells to enzalutamide.

Inhibition of GR is required for sensitivity to ipatasertib in the context of GR-dependent tumor growth

To confirm whether resensitization of cells to enzalutamide by ipatasertib treatment was through inhibition of GR, we genetically knocked down *NR3C1* in LREX cells using shRNA (Supplementary Fig. S4A) and treated two independent *NR3C1*-KD lines with enzalutamide. Our data demonstrate that when lacking GR, LREX cells are not robust when grown in the absence of androgen (DMSO alone), with further decreased viability with the addition of enzalutamide and/or ipatasertib (**Fig. 3E**).

Next, we sought to determine whether decreased GR expression is required for sensitivity to ipatasertib. We maintained GR expression in LREX and LNCaP cells by transfecting them with a GR-expression construct (Supplementary Fig. S4B, left). LNCAP cells with exogenous expression of GR demonstrated resistance to ipatasertib compared with the control, as well as resistance to enzalutamide and combination treatment (**Fig. 3F**). LREX with exogeneous expression of GR are also resistant to ipatasertib (Supplementary Fig. S4B, right). In contrast to LNCaP and LREX, the 22RV1 cell line has high *NR3C1* mRNA and GR protein expression at the basal level and lacks further induction of GR expression following AR inhibition (24). GR protein level is maintained in the presence of ipatasertib, and the cell line is minimally sensitive to ipatasertib (Supplementary Fig. S4C). This suggests that the effect of ipatasertib on GR expression and cell viability is enhanced when GR is induced by AR inhibition, but not when there is constitutive high expression. Overall, our data suggest that AKT inhibition blocks the induction of GR, reducing cell viability and increasing sensitivity to AR blockade.

Impact of AKT inhibition on GR expression is mediated through AR

We have demonstrated blockade of GR expression at the protein and transcript level using several PI3K/AKT pathway inhibitors, including ipatasertib (**Fig. 3D**). Interestingly, exogenous androgen (DHT) also suppressed *NR3C1* expression, whereas *NR3C1* expression was increased in the presence of enzalutamide. PI3K/AKT pathway signaling was previously shown to suppress AR activity, and AR is thought to negatively regulate transcription of *NR3C1* (9). To determine whether AKT inhibition suppresses GR expression and activity through AR-mediated regulation of transcription, we determined the effect of ipatasertib on canonical AR activity. GSEA was performed using RNA-seq to identify gene sets that were significantly enriched or depleted with each treatment condition. We found among MSigDB Hallmark gene sets, the most upregulated in any condition was androgen response following exposure to ipatasertib (**Fig. 4A** and **B**; Supplementary Fig. S5). We further examined the expression of a panel of AR-regulated genes following exposure to ipatasertib or enzalutamide. Whereas enzalutamide decreased the expression of AR-regulated genes, ipatasertib caused a mixed response where, on average, the expression of AR target genes was increased (Supplementary Fig. S6A). In addition, we examined AR activity using a luciferase reporter driven by tandem AR response elements. While ipatasertib alone drove similar expression to control, enzalutamide markedly decreased luciferase expression, which was reversed by adding in ipatasertib (Supplementary Fig. S6B).

To more broadly examine the effect of ipatasertib exposure on transcription activity, we looked at transcription factor motif enrichment. Motif analysis in H3K27ac-decorated chromatin identified enrichment in samples treated with ipatasertib, both alone and in combination with enzalutamide, of transcription factor sequences recognized by the AR (**Fig. 4C**). This included substantial deposition of H3K27ac at several canonical AR target loci, including the *KLK3* superenhancer locus, which was increased by ipatasertib and decreased by enzalutamide (**Fig. 4D**). RNA-seq experiments performed in parallel indicate increased transcription of canonical AR targets *KLK3* and *TMPRSS2* with ipatasertib treatment (**Fig. 4E**).

To further evaluate the direct effect of AR on GR expression at the transcript level, we genetically knocked down the AR gene in LREX cells. We selected two of four target sequences that produced >70% knockdown for our experiments (Supplementary Fig. S6C). We demonstrate that in both AR knockdown models, there was an increase in *NR3C1* expression compared with the nonsilencing control, confirming that AR activity directly influences GR expression (Supplementary Fig. S6D). In addition, we evaluated *NR3C1* expression in both nonsilencing and AR knockdown cells following exposure to ipatasertib. As expected, *KLK3* expression was increased with ipatasertib treatment in nonsilencing controls, but AR knockdown cells had very low *KLK3* expression regardless of treatment condition (**Fig. 4F**). Importantly, nonsilencing control cells had a decrease in *NR3C1* expression with ipatasertib treatment, which is eliminated when AR is knocked down. In fact, there was an increase in *NR3C1* expression in this setting. Taken together, these data indicate that AKT inhibition activates canonical AR targets, which in turn blocks GR induction, through *cis*-regulatory elements that sense the depletion in PI3K–AKT signal.

Combination AKT inhibition and androgen inhibition decreases tumor size across multiple xenograft models

Having established that PI3K/AKT inhibition blocks GR induction to suppress cancer cell growth *in vitro* and cooperates with AR inhibition to induce cancer cell death, we next sought to evaluate the effect of ipatasertib on established tumors *in vivo*, either alone or in the setting of AR-targeted therapy. We used two independent PDX models to establish tumors in immunocompromised mice and measured tumor volume following treatment with ipatasertib and/or castration. These models represent a range of AR activity and AR-responsiveness and baseline GR expression (Supplementary Fig. S7; ref. 21). When tumors were well established ($\sim$500 mm^3), the mice were castrated, given ipatasertib by oral gavage daily (5 days on; 2 days off), or both. In both models, ipatasertib halted tumor growth as well as or better than castration (average relative change compared with pretreatment with ipatasertib, -0.3632 vs. control 0.6591; $P = 0.002$; **Fig. 5A**). Moreover, castration and ipatasertib combined consistently led to decreases in tumor volume in each model (average relative change compared with pretreatment in combination, -2.737 vs. control, ipatasertib and castration 0.4069; $P = 0.0004$; **Fig. 5A**).

We additionally tested ipatasertib and AR inhibition in two models of CRPC. The LuCaP 136CR-N model was derived *in vivo* from the parental LuCaP 136 model grown in castrated mice. We also examined xenografts grown from the LREX cell line. Castrated mice harboring tumors from these CRPC models were exposed to ipatasertib, enzalutamide, or both. In both CRPC models, enzalutamide had little effect,

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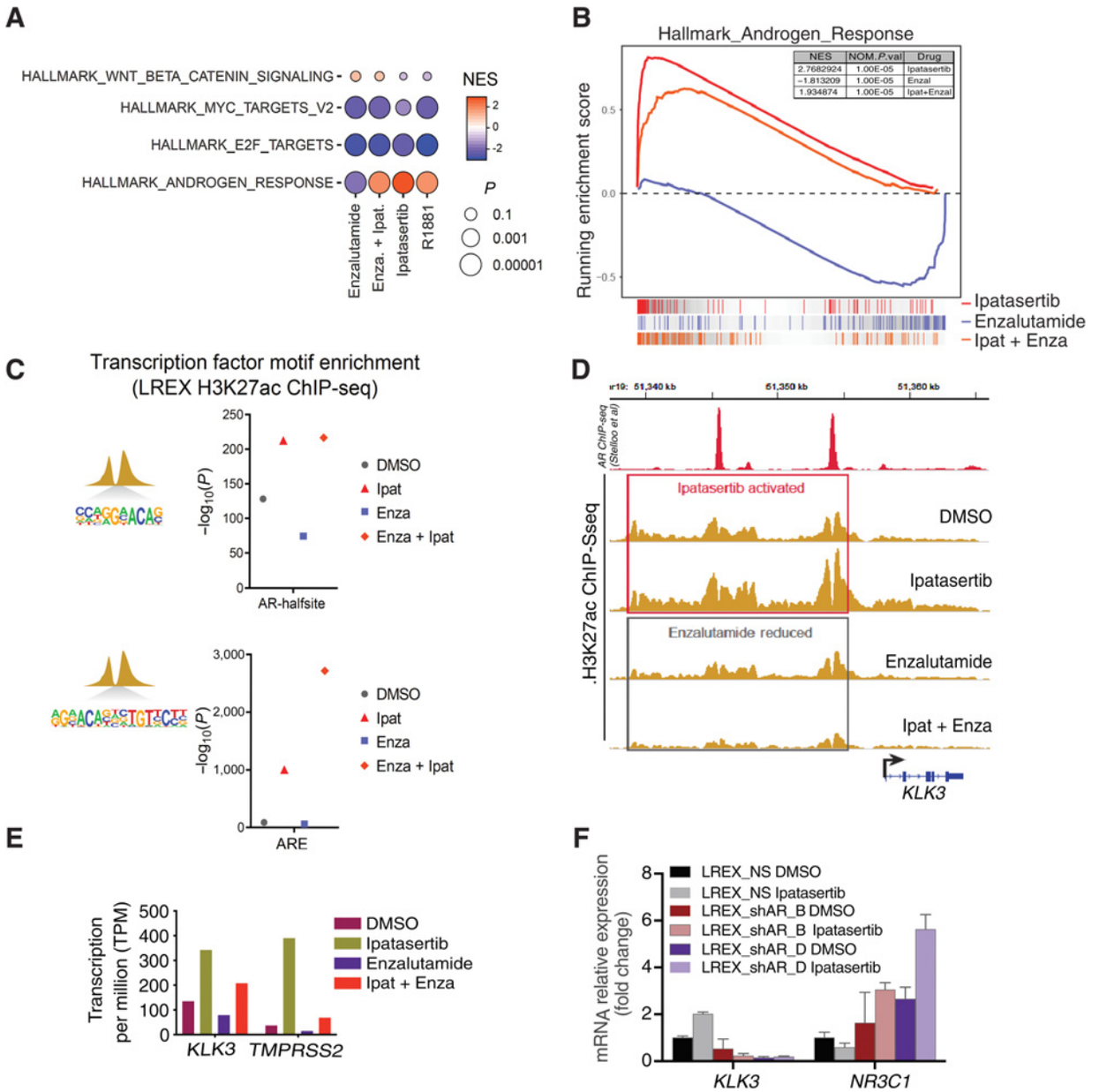

Figure 4.

Blockade of GR following ipatasertib exposure is modulated by AR. **A,** Bubble lattice plot of selected enriched hallmark pathways (also see Supplementary Fig. S4). **B,** GSEA comparing gene expression of LREX cells treated with ipatasertib or combination show positive enrichment of genes associated with androgen response and negative enrichment with enzalutamide. **C,** Motif enrichment plots for H3K27ac sites indicates enrichments for AR-half sites and AREs in active promoters and enhancers in LREX cells following exposure to ipatasertib alone or with enzalutamide. **D,** H3K27ac ChIP-seq aligned to published AR ChIP-seq track experiments performed on LREX cells 48 hours posttreatment indicates diminishing of enhancer deposits at the KLK3 locus in cells treated with enzalutamide or combination of both ipatasertib and enzalutamide. **E,** Gene expression data from RNA-seq experiments demonstrates increased expression of the AR-regulated genes *KLK3* and *TMPRSS2*. **F,** Increase in KLK3 is observed in nonsilencing control cells following exposure to ipatasertib, but unchanged in AR knockdown cells, as expected (left). qRT-PCR indicates decreased *NR3C1* gene expression in the presence of ipatasertib, which is increased with ipatasertib in engineered cells compared with the nonsilencing control (right). Graphs are presented as mean ± SD.

with tumor growth rates similar to that of controls (average relative change compared with pretreatment in enzalutamide, 0.5180 vs. control, 0.5844; $P = 0.99$; **Fig. 5B**). Ipatasertib, however, caused a dramatic slowing of tumor growth or a decrease in tumor volume (average relative change compared with pretreatment with ipatasertib, −14.95 vs. control and enzalutamide; $P = 0.0003$). The combination of enzalutamide and ipatasertib showed similar results (average relative change compared with pretreatment with combination, −23.15 vs. control and enzalutamide; $P = 0.0001$; **Fig. 5B**). Thus, there is a consistent antitumor effect of AKT inhibition in diverse *in vivo*

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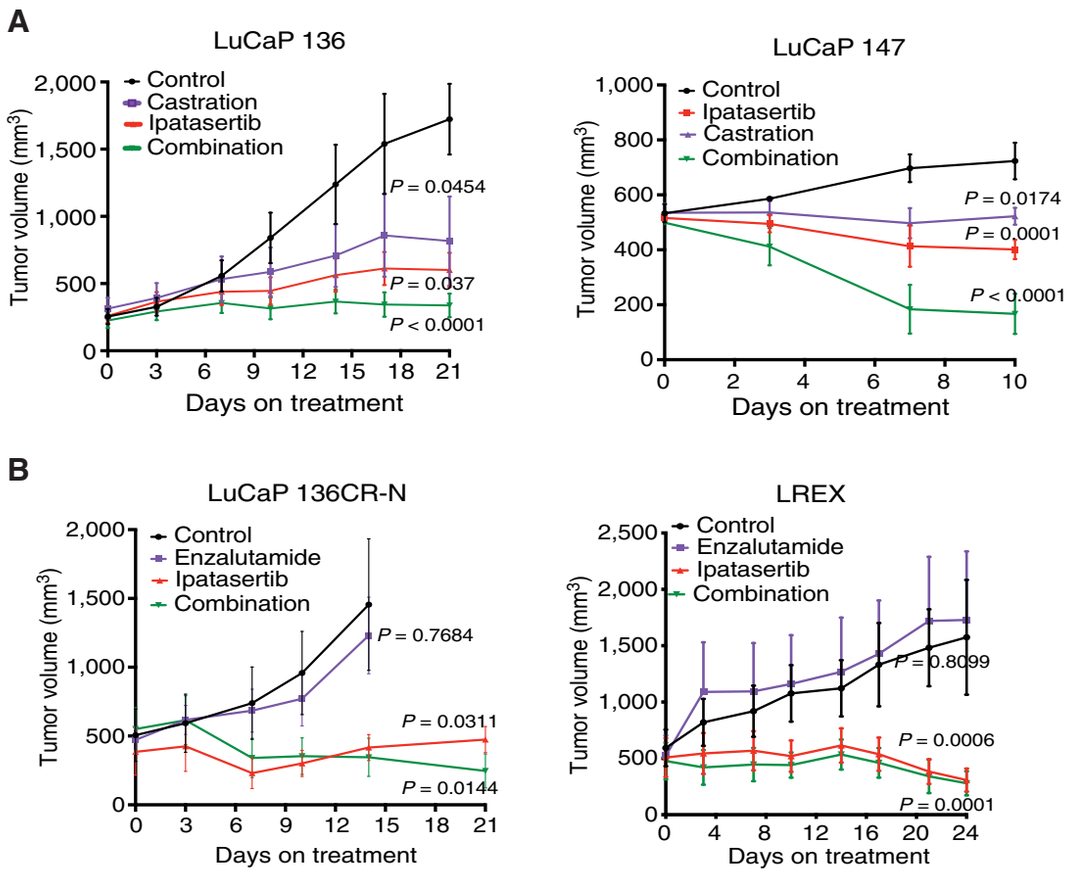

Figure 5.

Ipatasertib and AR inhibition combine to inhibit tumor growth in *in vivo* prostate cancer models. **A,** In models of androgen-sensitive prostate cancer, LuCaP 147 and LuCaP 136 grown in intact mice, ipatasertib decreased tumor burden as monotherapy, with enhanced efficacy when combined with castration. **B,** In enzalutamide-resistant prostate cancer, LuCaP 136CR-N PDX and LREX grown in castrated mice, ipatasertib effectively inhibits tumor growth as a single agent and in combination with enzalutamide. Graphs are presented as the mean ± SD. (n = 7–8/group for LuCaP 136, 136CR and LREX, n = 6/group for LuCaP 147).

prostate cancer models, representing both hormone sensitive and resistant disease.

AKT inhibition enhances AR activity and decreases GR expression *in vivo*

Having demonstrated in cell line models that PI3K/AKT pathway inhibitors block GR expression, and that this regulation of GR expression is in part through increased AR activity, we sought to confirm these findings in mouse xenograft models. We took EOT tumors from experiments shown in **Fig. 6** and evaluated mRNA and/or protein of GR- and AR-regulated genes. Similar to *in vitro* experiments, castration or enzalutamide led to a marked decrease of AR activity, as indicated by decreased expression of downstream genes *KLK3* and *NKX3-1*, whereas ipatasertib caused increased expression of these genes (**Fig. 6A–D**). Ipatasertib caused concomitant decrease in GR protein level, which was maintained or increased with castration (**Fig. 6E**). Similar results were seen in CRPC models treated with enzalutamide (**Fig. 6F**). These effects were maintained throughout treatment, as tumors were harvested after 2 or 4 weeks of therapy.

There is evidence of a similar sustained increase in GR activity in patients with mCRPC. In evaluating RNA-seq data from 212 tumor specimens from patients with mCRPC (30), tumors from patients that were currently on abiraterone and/or enzalutamide had increased expression of a gene signature reflecting GR activity, with some overlap with AR activity (GR1; Supplementary Fig. S8). When the signature was narrowed to more specifically reflect GR activity alone (GR2), it was also increased in those who had previously progressed on such therapy.

Discussion

The PI3K/AKT pathway is a complex signaling pathway that is involved in survival, proliferation, metabolism, and growth pathways. In this study, we show that inhibition of the PI3K/AKT signaling pathway can block GR expression that is induced as a resistance mechanism to AR-targeted therapy, both when combined with androgen deprivation or the AR antagonist enzalutamide. In multiple prostate cancer xenograft models representing a spectrum of AR-dependence, this leads to significant antitumor response. Blockade of GR expression is mediated through induction of canonical AR activity, and it is associated with reorganization of the chromatin landscape and decreased expression of the GR gene *NR3C1* (**Fig. 6G**).

While multiple mechanisms can lead to resistance to AR-targeted therapy, one that has drawn particular interest is the induced expression of GR, leading to a new therapeutic strategy for a subset of patients with prostate cancer. Interestingly, the upregulation of GR as

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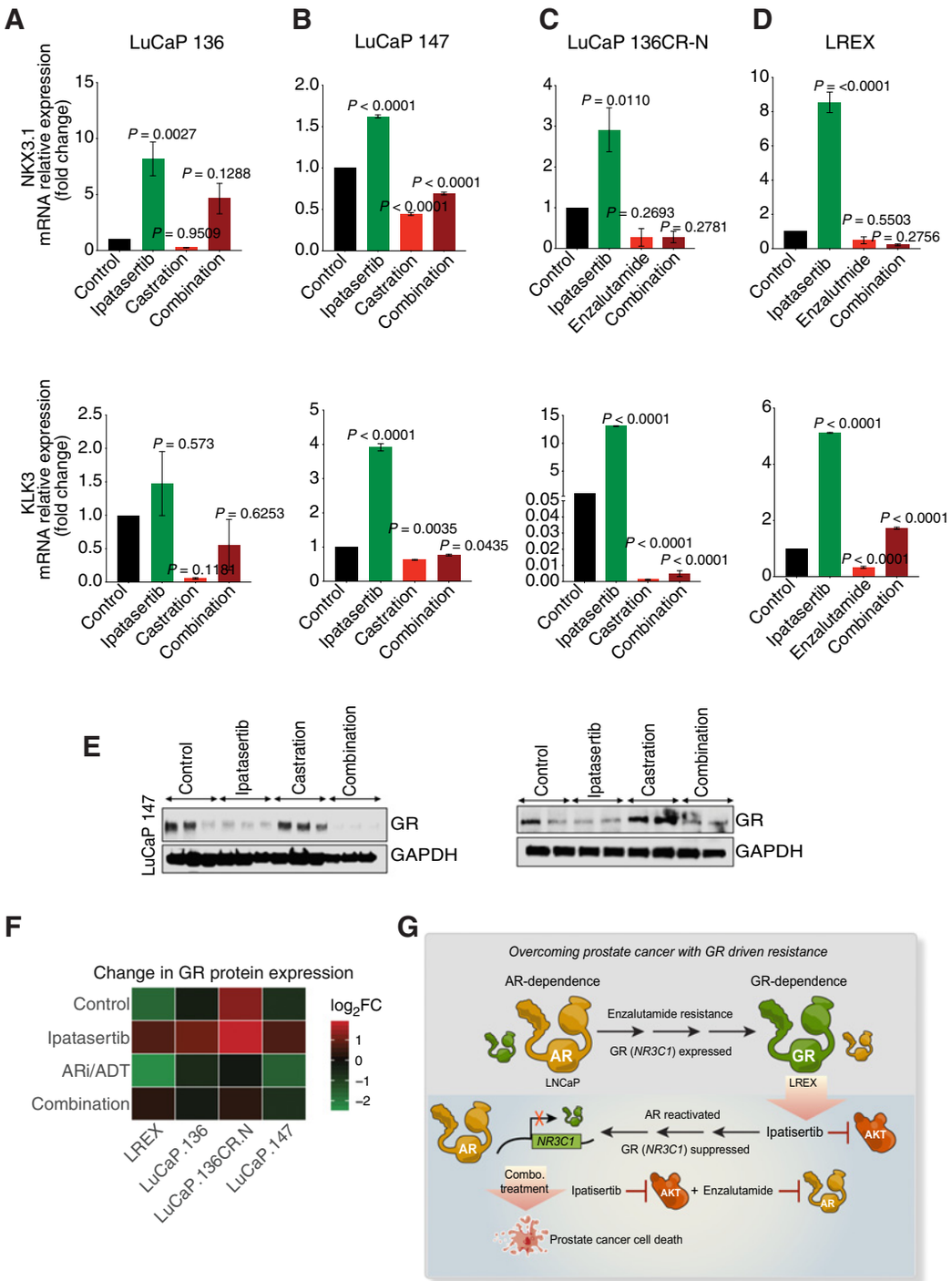

Figure 6.

AKT inhibition enhances AR activity and blocks GR expression *in vivo*. **A–D,** RT-PCR from three PDXs and one cell line treated with the pan-AKT inhibitor ipatasertib (100 mg by oral gavage), castration, enzalutamide or combination of ipatasertib + castration/ipatasertib + enzalutamide. As expected, expression of canonical AR-regulated genes *KLK3* (PSA) and *NKX3-1* are decreased with castration or enzalutamide. Expression of these genes is increased with ipatasertib or with enzalutamide combined with ipatasertib. **E,** Representative Western blot analysis from LuCaP 136 and LuCaP 147 showing increased GR expression following castration, and decreased levels after exposure to ipatasertib. **F,** Summary heatmap of all models shows the expression of GR protein is decreased with ipatasertib, which is attenuated modestly in castrated/enzalutamide-treated animals in most models. **G,** Model of regulation of GR by AKT inhibition in the context of GR-dependent growth. AKT inhibition decreases GR at the transcriptional level through induction of AR activity. Graphs are presented as mean ± SEM.

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compensatory hormone receptor signaling has also been reported in breast cancer and is associated with poor prognosis in triple-negative breast cancer (31, 32). It is likely that diverse mechanisms can regulate GR expression in diverse prostate cancer subtypes. Indeed, 22Rv1 cells, which express constitutively high levels of GR that is neither affected by AR inhibition nor ipatasertib, are relatively resistant to ipatasertib. Nevertheless, we demonstrate here that ipatasertib blocks the induction of GR expression across numerous models tested, both *in vitro* and *in vivo*, which is associated with ipatasertib sensitivity. In two engineered GR-overexpressing cells lines, we show enforced GR expression reduces ipatasertib sensitivity, demonstrating the key role of GR expression and activity in response and resistance to AKT inhibition. To our knowledge, this is the first demonstration that PI3K/AKT pathway inhibition blocks GR expression in prostate cancer, leading to significant antitumor effect.

The strongest evidence to date for upregulation of GR is in response to enzalutamide for mCRPC (8, 9, 24, 33). In advanced prostate cancer, ipatasertib has been used in combination with abiraterone, which is given with prednisone, providing further rationale for blockade of GR contributing to antitumor effect, and we give evidence from patient data that a significant portion of patients with mCRPC have sustained induction of GR activity following exposure to AR pathway inhibitors. The importance of GR expression for development of castrate resistance in newly diagnosed metastatic disease in response to initial therapy, whether with ADT alone, ADT and docetaxel, or ADT and an AR pathway inhibitor (abiraterone/prednisone, enzalutamide, or apalutamide), has not been investigated. It is worth noting that we found marked antitumor effect with ipatasertib monotherapy and in combination with castration in multiple PDX models of HSPC. In some respects, high-risk localized disease may have similar biology to metastatic HSPC. Upregulation of GR expression has been demonstrated in a subset of patients in both neoadjuvant enzalutamide and neoadjuvant abiraterone/prednisone trials in this setting (34, 35), raising the question of whether adding a PI3K/AKT inhibitor could improve the outcomes for those with HSPC. One explanation for the prevalence of GR expression in these early-phase studies, as opposed to well-characterized genomic alterations (e.g., AR enhancer amplification), is that development of resistance through selection of genomically altered subclones may require more time, and later-stage disease, than induction of GR.

In our studies, regulation of GR induction by the PI3K/AKT pathway is associated with remodeling of the chromatin landscape. It is likely that there are additional important consequences of chromatin remodeling aside from regulation of GR expression and activity. The consistent effects on GR across diverse models, however, and the requirement for GR inhibition for maximal inhibitor sensitivity, suggest that the effect on the *NR3C1* gene and GR activity may be crucial. The PI3K/AKT pathway has been demonstrated to have effects on the chromatin landscape in breast cancer, as well, another hormone receptor–regulated cancer (36). The full consequences of AKT inhibition on chromatin remodeling in prostate cancer have yet to be elucidated.

Along with inhibition of GR, AKT inhibition induces an increase in canonical AR activity. This seems counterintuitive because many prostate cancer therapies are designed to inhibit AR activity. Yet others have noted in tumor samples that high AR activity is associated with low cell proliferation (37). It is not clear whether this is the same mechanism behind responses induced by supraphysiologic testosterone in recent clinical trials (38, 39). It may be that increased canonical activity is associated with decreased noncanonical activity. More extensive studies will be required to tease out the paradoxical mechanisms behind different levels of AR activity.

It has been suggested that PI3K/AKT-targeted therapies might drive differentiation toward t-SCNC, as demonstrated with the LNCaP cell line (40). In our data, however, a responsive PDX model, LuCaP 136, represents aggressive variant prostate cancer due to loss of function of both PTEN and TP53 (41). Indeed, others have demonstrated that activated AKT and N-MYC combine to drive a neuroendocrine phenotype (42), supporting the use of an AKT inhibitor for tumors with neuroendocrine features.

This study was focused on prostate cancer, for which induction of GR expression is a demonstrated mechanism of resistance for established therapies. PI3K pathway inhibition in general, and AKT inhibition with ipatasertib in particular, have been and continue to be tested in a number of other solid tumors. In breast cancer, GR expression has been shown to play a role in resistance to taxanes, with apparently opposite effects in hormone receptor–positive and hormone receptor–negative disease (31). It is not known whether ipatasertib blocks GR activity in breast cancer as it does in prostate cancer, or if that plays a role in the efficacy seen in a recent phase II study (16).

The long history of PI3K/AKT pathway inhibitors for the treatment of prostate cancer and other solid tumors has translated to only modest success in the clinic. The pan-AKT inhibitor ipatasertib shows promise in combination with abiraterone plus prednisone for late-stage prostate cancer. Our data demonstrate marked antitumor effect in models that upregulate GR to induce resistance. A better understanding of the clinical settings in which GR activity is most critical will help usher in a new target in the PCa therapy armamentarium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Adelaiye-Ogala, D.J. VanderWeele
Development of methodology: R. Adelaiye-Ogala, D.J. VanderWeele
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Adelaiye-Ogala, Y.T.M. Nguyen, A.N. Alilin, A.R. Grayson, K.H. Jansson, M.L. Beshiri, J.A. Rodriguez-Nieves, D.J. VanderWeele
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Adelaiye-Ogala, B.E. Gryder, A.R. Grayson, K.H. Jansson, M.L. Beshiri, S. Agarwal, B. Capaldo, K. Kelly, D.J. VanderWeele
Writing, review, and/or revision of the manuscript: R. Adelaiye-Ogala, B.E. Gryder, S. Agarwal, B. Capaldo, K. Kelly, D.J. VanderWeele
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Adelaiye-Ogala, W. Bajwa
Study supervision: K. Kelly, D.J. VanderWeele

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References

1. Polotti CF, Kim CJ, Chuchvara N, Polotti AB, Singer EA, Elsamra S. Androgen deprivation therapy for the treatment of prostate cancer: a focus on pharmacokinetics. Expert Opin Drug Metab Toxicol 2017;13:1265–73.
2. Virgo KS, Basch E, Loblaw DA, Oliver TK, Rumble RB, Carducci MA, et al. Second-line hormonal therapy for men with chemotherapy-naive, castration-resistant prostate cancer: American Society of Clinical Oncology Provisional Clinical Opinion. J Clin Oncol 2017;35:1952–64.
3. Loblaw DA, Virgo KS, Nam R, Somerfield MR, Ben-Josef E, Mendelson DS, et al. Initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer: 2006 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol 2007;25:1596–605.
4. Takeda DY, Spisak S, Seo JH, Bell C, O'Connor E, Korthauer K, et al. A somatically acquired enhancer of the androgen receptor is a noncoding driver in advanced prostate cancer. Cell 2018;174:422–32.
5. Crona DJ, Milowsky MI, Whang YE. Androgen receptor targeting drugs in castration-resistant prostate cancer and mechanisms of resistance. Clin Pharmacol Ther 2015;98:582–9.
6. Chism DD, De Silva D, Whang YE. Mechanisms of acquired resistance to androgen receptor targeting drugs in castration-resistant prostate cancer. Expert Rev Anticancer Ther 2014;14:1369–78.
7. Efstathiou E, Titus M, Wen S, Hoang A, Karlou M, Ashe R, et al. Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer. Eur Urol 2015;67:53–60.
8. Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell 2013;155:1309–22.
9. Xie N, Cheng H, Lin D, Liu L, Yang O, Jia L, et al. The expression of glucocorticoid receptor is negatively regulated by active androgen receptor signaling in prostate tumors. Int J Cancer 2015;136:E27–38.
10. Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell 2017;169:381–405.
11. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN- deficient prostate cancer. Cancer Cell 2011;19:575–86.
12. Blake JF, Xu R, Bencsik JR, Xiao D, Kallan NC, Schlachter S, et al. Discovery and preclinical pharmacology of a selective ATP-competitive Akt inhibitor (GDC-0068) for the treatment of human tumors. J Med Chem 2012;55:8110–27.
13. Yan Y, Serra V, Prudkin L, Scaltriti M, Murli S, Rodriguez O, et al. Evaluation and clinical analyses of downstream targets of the Akt inhibitor GDC-0068. Clin Cancer Res 2013;19:6976–86.
14. de Bono JS, De Giorgi U, Rodrigues DN, Massard C, Bracarda S, Font A, et al. Randomized phase II study evaluating Akt blockade with ipatasertib, in combination with abiraterone, in patients with metastatic prostate cancer with and without PTEN loss. Clin Cancer Res 2019;25:928–36.
15. de Bono JS, De Giorgi U, Nava Rodrigues D, Massard C, Bracarda S, Font A, et al. Randomized phase II study of Akt blockade with or without ipatasertib in abiraterone- treated patients with metastatic prostate cancer with and without PTEN loss. Clin Cancer Res 2019;25:928–36.
16. Kim SB, Dent R, Im SA, Espie M, Blau S, Tan AR, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2017;18:1360–72.
17. Trials GC. Explore Currently Enrolling Genentech and Roche Clinical Trials in Oncology; 2018. Available from: http://www.genentechclinicaltrials.com.
18. Beshiri ML, Tice CM, Tran C, Nguyen HM, Sowalsky AG, Agarwal S, et al. A PDX/organoid biobank of advanced prostate cancers captures genomic and phenotypic heterogeneity for disease modeling and therapeutic screening. Clin Cancer Res 2018;24:4332–45.
19. Adelaiye-Ogala R, Damayanti NP, Orillion AR, Arisa S, Chintala S, Titus MA, et al. Therapeutic targeting of sunitinib-induced AR phosphorylation in renal cell carcinoma. Cancer Res 2018;78:2886–96.
20. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 2014;159:1665–80.
21. Nguyen HM, Vessella RL, Morrissey C, Brown LG, Coleman IM, Higano CS, et al. LuCaP prostate cancer patient-derived xenografts reflect the molecular heterogeneity of advanced disease and serve as models for evaluating cancer therapeutics. Prostate 2017;77:654–71.
22. Mulholland DJ, Tran LM, Li Y, Cai H, Morim A, Wang S, et al. Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell 2011;19:792–804.
23. Szmulewitz RZ, Chung E, Al-Ahmadie H, Daniel S, Kocherginsky M, Razmaria A, et al. Serum/glucocorticoid-regulated kinase 1 expression in primary human prostate cancers. Prostate 2012;72:157–64.
24. Isikbay M, Otto K, Kregel S, Kach J, Cai Y, Vander Griend DJ, et al. Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. Horm Cancer 2014;5:72–89.
25. Heintzman ND, Ren B. Finding distal regulatory elements in the human genome. Curr Opin Genet Dev 2009;19:541–9.
26. Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 2010;107:21931–6.
27. Gryder BE, Yohe ME, Chou HC, Zhang X, Marques J, Wachtel M, et al. PAX3-FOXO1 establishes myogenic super enhancers and confers BET bromodomain vulnerability. Cancer Discov 2017;7:884–99.
28. Zhang Z, Chng KR, Lingadahalli S, Chen Z, Liu MH, Do HH, et al. An AR-ERG transcriptional signature defined by long-range chromatin interactomes in prostate cancer cells. Genome Res 2019;29:223–35.
29. Dowen JM, Fan ZP, Hnisz D, Ren G, Abraham BJ, Zhang LN, et al. Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. Cell 2014;159:374–87.
30. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci U S A 2019;116:11428–36.
31. Pan D, Kocherginsky M, Conzen SD. Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. Cancer Res 2011;71:6360–70.
32. Skor MN, Wonder EL, Kocherginsky M, Goyal A, Hall BA, Cai Y, et al. Glucocorticoid receptor antagonism as a novel therapy for triple-negative breast cancer. Clin Cancer Res 2013;19:6163–72.
33. Puhr M, Hoefer J, Eigentler A, Ploner C, Handle F, Schaefer G, et al. The glucocorticoid receptor is a key player for prostate cancer cell survival and a target for improved antiandrogen therapy. Clin Cancer Res 2018;24:927–38.
34. Sowalsky AG, Ye H, Bhasin M, Van Allen EM, Loda M, Lis RT, et al. Neoadjuvant-intensive androgen deprivation therapy selects for prostate tumor foci with diverse subclonal oncogenic alterations. Cancer Res 2018;78:4716–30.
35. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447–54.
36. Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, et al. PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. Science 2017;355:1324–30.
37. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med 2016;22:369–78.
38. Schweizer MT, Antonarakis ES, Wang H, Ajiboye AS, Spitz A, Cao H, et al. Effect of bipolar androgen therapy for asymptomatic men with castration-resistant prostate cancer: results from a pilot clinical study. Sci Transl Med 2015;7:269ra2.
39. Lam HM, Nguyen HM, Labrecque MP, Brown LG, Coleman IM, Gulati R, et al. Durable response of enzalutamide-resistant prostate cancer to supraphysiological testosterone is associated with a multifaceted growth suppression and impaired DNA damage response transcriptomic program in patient-derived xenografts. Eur Urol 2020;77:144–55.
40. Chen R, Li Y, Buttyan R, Dong X. Implications of PI3K/AKT inhibition on REST protein stability and neuroendocrine phenotype acquisition in prostate cancer cells. Oncotarget 2017;8:84863–76.
41. Aparicio AM, Shen L, Tapia EL, Lu JF, Chen HC, Zhang J, et al. Combined tumor suppressor defects characterize clinically defined aggressive variant prostate cancers. Clin Cancer Res 2016;22:1520–30.
42. Lee JK, Phillips JW, Smith BA, Park JW, Stoyanova T, McCaffrey EF, et al. N-Myc drives neuroendocrine prostate cancer initiated from human prostate epithelial cells. Cancer Cell 2016;29:536–47.

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Molecular Cancer Therapeutics

Targeting the PI3K/AKT Pathway Overcomes Enzalutamide Resistance by Inhibiting Induction of the Glucocorticoid Receptor

Remi Adelaiye-Ogala, Berkley E. Gryder, Yen Thi Minh Nguyen, et al.

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Alterations in AR regulome following response to kinase inhibitors

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The androgen receptor (AR) signaling axis is critical for prostate cancer (PCa) pathogenesis and all subsequent phases of disease progression. Despite initial success with androgen deprivation therapy and/or AR targeted agents such as enzalutamide or abiraterone, most patients inevitably relapse due to therapeutic resistance. Previous studies have shown that despite resistance to AR antagonist, genomic AR is unchanged and its gene expression, unaltered. Therapeutic resistance has been broadly classified into two categories; restoration of AR activity and tumor progression despite AR blockade. Indeed, others have noted in clinical and preclinical specimens, an extensive reprogramming of the AR cistrome, where a shift in canonical to a non-canonical AR cistrome is associated with increase in Gleason grade. In previous studies, we observed increases in known AR target genes following exposure to kinase inhibitors which was marked with decrease in tumor growth. We hypothesized that restoration of canonical AR cistrome can be mediated through kinase inhibition and is associated with re-sensitization to AR targeted therapy. We treated cell lines and patient derived xenograft models of advanced prostate cancer with kinase signaling pathway inhibitors, AR signaling inhibitors or combination of both for a duration of 24 hours (*in vitro*) or 5 days (*in vivo*). Samples were and collected and processed for RNA-seq and ChIP-seq. From our transcriptomic analysis, we found enrichment of previously published non-canonical AR target genes in cells resistant to AR targeted agents. When resistant cells were exposed to kinase inhibitors, we observed an enrichment of canonical AR target genes. In addition, we identified unique AR enriched peaks at active sites associated with resistance and through GSEA of gene annotated peaks, we found unique regulatory transcription factor targets some of which are associated with cell proliferation and differentiation and may be playing a functional role on AR cistrome adaptation to resistance and survival. Overall, our data so far suggests that AR transcription adapts to therapeutic resistance of AR antagonist via enrichment of non-canonical AR cistrome. Furthermore, restoration of canonical AR cistrome is achieved following kinase signaling pathway inhibition and is associated with re-sensitization to AR targeted therapy.

Dynamics of Epigenetic Mechanisms in the Akt Signaling Pathway and Its Influences on Drug Response in Advanced Prostate Cancer

PC180871

W81XWH-19-1-0715

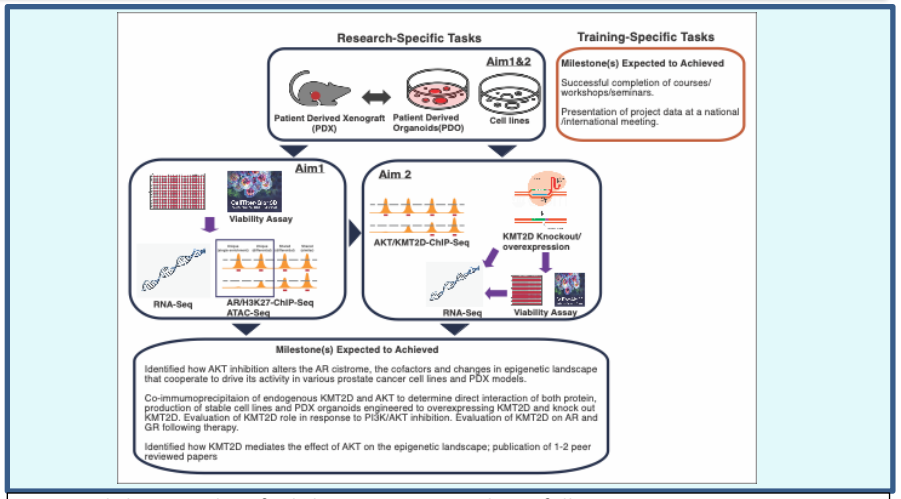
PI: Dr. Remi Adelaiye-Ogala **Org:** The Geneva Foundation **Award Amount:** $239,600.00

Study/Product Aim(s)

- Aim 1 will be focused on performing comprehensive analysis identifying changes in AR cistrome and the broader epigenetic landscape after exposure to PI3K/AKT pathway targeted therapies.
- Aim 2 will be focused on performing mechanistic studies to test the hypothesis that PI3K/AKT signaling alters AR activity and cofactor binding through KMT2D.

Approach

- Approach to achieving aim 1: Identifying of changes in AR binding and activity following exposure to PI3K/AKT pathway-targeted therapy followed by in-depth profiling of changes in the epigenome in relation to AKT signaling.
- Approach to achieving aim 2: Using KMT2D overexpressing or knock-out models to assess its link with AKT and evaluate the effects on AR regulome.



Accomplishment: Identified changes in AR regulome following exposure to an AKT inhibitor. Published 1 peer-reviewed manuscript. Completed training workshops, seminars and presented work at a national scientific retreat.

Timeline and Cost

Activities CY	19-20	20-21	21-22	
Training and educational development in prostate cancer research				
Identification of changes in AR binding and activity following exposure to PI3K/AKT pathway-targeted therapies				
To perform in-depth profiling of changes in the epigenome in relation to AKT signaling inhibition				
Evaluate the effects of KMT2D overexpression or knock-out on response to PI3K/AKT inhibition including anti- tumor effect, AR activity, and GR expression				
Estimated Budget ($K)	**$177,684**	**$61,916**		

Updated: (10/11/2021)

Goals/Milestones (Example)

CY19 Goal – Completed training workshops/seminars. Identified changes in AR regulome following exposure to an AKT inhibitor. Published 1 peer-reviewed manuscript,

☑

CY20 Goals – Identified changes in the epigenome following AKT signaling inhibition. Present research work at national and international scientific meetings

☑

CY21 Goal – Transitioned to role of an independent investigator in the field of prostate cancer.

☐

CY22 Goal – Determine the link of histone methyltransferase, KMT2D and AKT signaling and evaluate the effects on AR regulome and the broader epigenetic landscape. 1-2 publications of results from this project.

☐

Comments/Challenges/Issues/Concerns

Due to the extensive period of lockdown as a result of the COVID-19 pandemic, we have re-evaluated the timeline of experiments to address all proposed aims. We anticipate changes in expenditures and cost of services due to these delays.

Budget Expenditure to Date

Projected Expenditure: $61,916.48

Actual Expenditure: $177,683.52