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14. ABSTRACT In year 2, we extended our studies to in vivo animal experiments. Three animal models, LNCaP C4-2B MDV-R, LNCaP GRP Pro cells and CWR22 xenograft were used. In LNCaP C4-2B MDV-R study, we validated that using autophagy modulators such as clomipramine and metformin, tumor survival mechanism encountering enzalutamide treatment may be blocked. A much significant reduction of tumor growth (91% and 78% for combinational use of CMI and metf with enzalutamide, respectively) was observed. In the LNCaP GRP model, combinational treatment of enzalutamide and saracatinib provided the best tumor suppression. Both specific mechanisms GRP cells utilizes for aberrant AR activation, intracrine androgen synthesis and kinase pathway mediated AR activation in the absence of androgen, were prevented through combined target therapy. Lastly, castration resistant gene activation and tumor recurrence was inhibited by saracatinib treatment in the CWR22 xenograft model but could be further enhanced when combined with autophagy modulator like metformin.					
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Introduction:

For our DOD award W81XWH-12-1-0529, we proposed to overcome autophagy as cell survival mechanism when treated with small molecule Src kinase inhibitor saracatinib or the androgen receptor signaling inhibitor enzalutamide in prostate cancer therapy. After the extensive in vitro studies of the effect of saracatinib and enzalutamide on CRPC cell lines, we have concluded that autophagy was the usual pathway for cancer cell survival. This treatment-mediated autophagy is through activation of AMPK and repression of mTOR [1-5]. Knocking down AMPK with siRNA reversed the survival mechanism and led cells to undergo apoptosis. Survival mechanisms elicited by CRPC C4-2B cells when treated with Enza may be blocked by inhibiting autophagy with clomipramine (CMI) [6, 7] and metformin (Metf) [8-10]. Combination of Enza with saracatinib and autophagy modulators significantly reduced cell proliferation in the LNCaP GRP CRPC model.

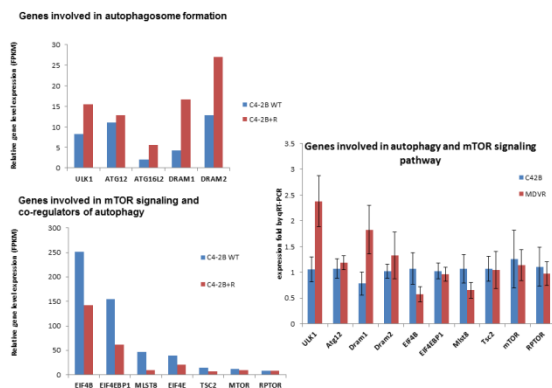


Figure 1. Transcriptome deep sequencing (left) of parental (WT) and enzalutamide resistant C4-2B (C4-2B+R) cells was carried out to examine differential gene expression pattern that may be related to their ability to survive under constant high exposure to ARSI. The data were validated with RT-qPCR (right).

parental cells (expression level are expressed in FPKM with 3 FPKM = 1 transcript/cell). We therefore attempted to knock down two of the autophagy central molecules Beclin 1 and Atg5 to examine the effect of modulating autophagy in anti-androgen treatments. Real-time qPCR was performed to validate the deep sequencing data and found the trend agreeable.

In the past year, we focused on in vivo studies using LNCaP C4-2B enza-resistant, GRP-Pro orthotopic and CWR22 xenograft models. With the former two CRPC models, combination of Enza with autophagy modulators CMI or Metf; or with saracatinib significantly reduced the tumor growth. The latter xenograft model showed that saracatinib alone may inhibit recurrence of castration resistant tumor growth after surgically castrated the CWR22 tumor bearing mice.

Keywords:

Castration resistant prostate cancer, autophagy, enzalutamide, saracatinib

Transcriptome deep sequencing of parental and Enza resistant C4-2B cells was carried out to examine differential gene expression pattern that may be related to their ability to survive under constant high exposure to ARSI. There were more than 140 genes up-regulated and more than 100 gene down-regulated, (3 FPKM = 1 transcript/cell). Figure 1 showed examples of up-regulated genes involved in autophagosome formation as expected in

the ENZA resistant cells. Suppression of mTOR signaling is also a major inducer of autophagy. We observed several mTOR signaling genes that were differentially down-regulated in the resistant cells when compared to the

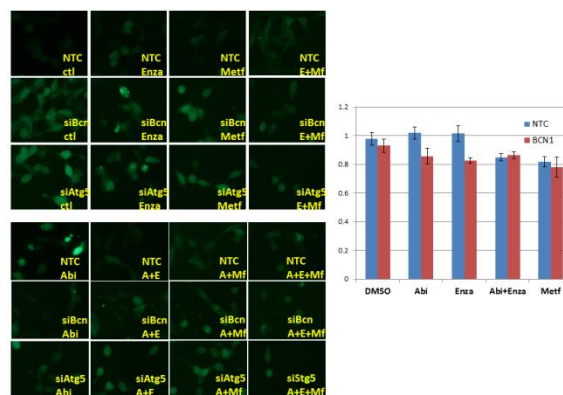


Figure 2. LNCaP LC3-GFP cells were transiently transfected with control (NTC), siRNA against beclin1 (Bcn) or Atg5, followed by treatments with DMSO, Enza, Metf, Abi or in combination. Autophagy represented by punctae of LC-3 was observed under fluorescence microscopy (left). MTT viability assay performed on the same setting showed the enhanced cell death after knocking out of Bcn1.

Overall Project Summary:

I. Combination of anti-androgen drugs and autophagy modulation (metformin or siRNA to Beclin 1 and Atg5) in LNCaP-eGFP-LC3 cells

LNCaP LC3-GFP cells were transiently transfected with control (NTC), siRNA against beclin1 (Bcn1) or Atg5 [11], followed by treatments with DMSO, Enza, Metf or in combination. Cells became sensitized to Enza and even more to Enza+metf after the autophagy genes were knocked down (Figure 2). Less punctae formation was detected in the siBcn or siAtg5 cells when treated with Enza. Metformin treatment on top of Enza further caused the morphology changes, cells were rounded up under the microscope indicating loss of viability. In additionally, they were treatments with abiraterone (Abi) or in combination with, Enza, Metf and both. Again, cells became sensitized to Abi and even more to combinations after the autophagy genes were knocked down. MTT assay was performed in C4-2B Enza-resistant cells with siRNA against beclin 1 followed by treatments with Abi, Enza, Metf or Abi+Enza. Anti-androgens alone did not inhibit growth in cells transfected with non-target control siRNA. However, cells with beclin1 knocked out became more susceptible to those drugs. Combination of Abi and Enza showed more potent effect even in the presence of autophagy pathway and so did metformin treatment. We are currently looking at the mechanisms behind.

II. Testing the combination of enzalutamide and CMI (or metformin) on LNCaP C42B MDVR orthotopic mouse model

To address our hypothesis that targeting autophagy could overcome resistant to ENZA therapy in CRPC in an in-vivo model, we used SCID mice and orthotopically implanted ENZA resistant cells into the prostate. PSA level was monitored until detectable around day 10, indicating tumor implantation. Treatments with

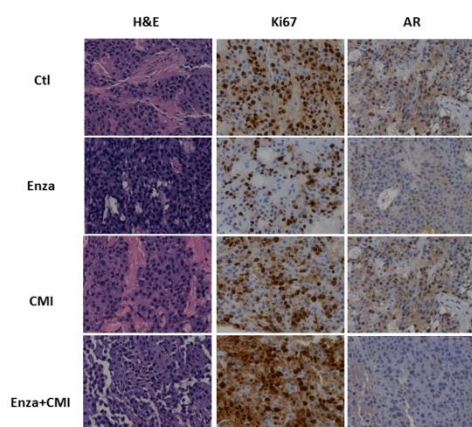


Figure 4. Tumors collected from different treatment groups, ctl, Enza, CMI and Enza+CMI were parafilm embedded and sectioned for H&E and immunohistochemical staining with antibodies against Ki67 or AR. The same analysis was done for the metformin group and got comparable results.

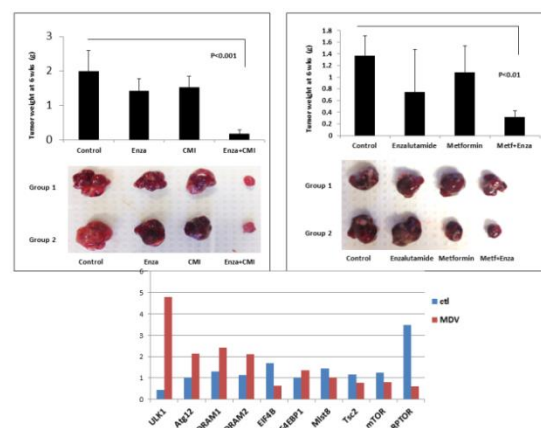


Figure 3. Two sets of orthotopic mouse studies were conducted with enzalutamide resistant C4-2B cells implanted into prostates of SCID mice. Drug treatments include enzalutamide, CMI, Metf or in combination. The graph in upper panels showed the average weight of tumors in each group, and two representative images of the tumors. RT-qPCR on genes involved in autophagy pathways were conducted on RNA extracted from respective tumor samples.

control vehicles, CMI, ENZA and combination were dosed daily. At the end of six weeks from surgery, tumors were harvested and weighed. Mice treated with ENZA or CMI alone showed a 28% and 23% decrease in tumor size when compared control mice, respectively. There were a significant reduction in tumor size by 91% in mice treated with ENZA in combination with the autophagy inhibitor, CMI when compared to control mice, as shown in Figure 3 ($p < 0.001$). Because the elevated interest of metformin also as an autophagy modulator and its low toxicity and applicability, we conducted another in vivo study replacing CMI with metformin. Mice treated with ENZA or metformin produced marginally reduced tumor sizes than the control mice, while those treated with the combination of ENZA and metformin gave a drastic 78% reduction with a significant difference ($p \leq 0.01$ by Student's T test). Tumors collected from different

Regulation of autophagy induction by the mTOR and AMPK complex

The diagram illustrates the regulation of autophagy induction by the mTOR and AMPK complex. It shows two main pathways: one involving AMPK and another involving mTOR.

AMPK Pathway: Starvation, Metformin, Genotoxic stress, Hypoxia, Anti Androgen, Enzalutamide, and Androgen deprivation activate AMPK. Activated AMPK (phosphorylated) inhibits the TSC2/TSC1 complex, which in turn inhibits Rheb. Rheb activates the mTORC1 complex (Raptor/mTORC1). mTORC1 phosphorylates ULK1/2, which inhibits the autophagy initiation complex (ATG-13, FIP-200). The mTORC1 complex also phosphorylates S6K1, which inhibits eIF4B and eIF4E. The mTORC1 complex also phosphorylates S6.

mTOR Pathway: Nutrients activate PI3K/AKT, which in turn activates Raptor/mTORC1. mTORC1 phosphorylates ULK1/2, which inhibits the autophagy initiation complex (ATG-13, FIP-200). The mTORC1 complex also phosphorylates S6K1, which inhibits eIF4B and eIF4E. The mTORC1 complex also phosphorylates S6.

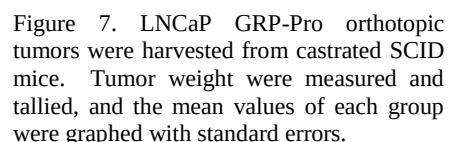
Autophagy: The autophagy initiation complex (ATG-13, FIP-200) leads to the formation of the autophagosome, which is then degraded by autophagy.

III. Testing the combination of inhibitory effect of saracatinib and enzalutamide on LNCaP GRP-Pro orthotopic mouse model

Serum PSA ng/ml						
control	saracatinib 25mg/d	Enzalutamide 30mg/d	sara+ENZA	intact control	castration control	
40.87	44.04	234.64	28.28	198.19	7.43	
200.15	214.45	246.57	60.28			
100.01	172.08	246.26	13.53			
187.23	163.25	5.52	5.35			
88.68	162.17	109.51	10.89			
197.89	112.51	26.25	5.33			
30.68	123.19	76.78	10.66			

Treatment Group	Mean Serum PSA (ng/ml)
control	~120
sara	~150
ENZA	~150
S+E	~20
intact	~200
castrated	~5

treatment groups, control, sara and ENZA, with the combination treatment group, sara+ENZA only 5/8 tumor take rate. The mean tumor weight in the combination group was 37% of that from the control group (Figure 7). The two monotherapy groups showed no inhibition and even increased tumor weight in average. The PSA serum levels in the first three groups were similar to that from an intact animal bearing prostate cancer tumors; whereas the combination group measured only 16% of the control. The immunohistochemical staining of representative samples clearly



showed inhibition of pSrc by sara and inhibition of AR nuclear translocation in sara treated groups (Figure 8).

IV. Preliminary testing of the combination of Src inhibitor saracatinib and chloroquine on the CWR22 xenograft model, the inhibition of recurrence and its gene analysis

CWR22 xenograft model [14, 15] was used to mimic tumor recurrence after androgen deprivation therapy in patients. Athymic Nu/Nu male mice were implanted with CWR22 xenograft subcutaneously and allow for tumor progression.

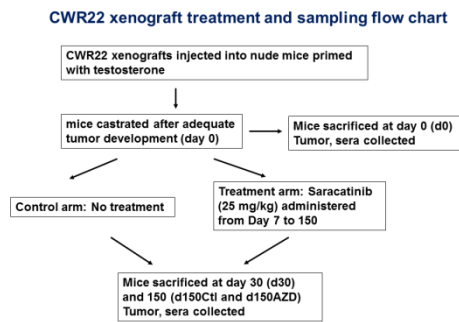


Figure 9. CWR22 xenograft treatment and sampling flow chart

treatment did not show significant tumor suppression after recurrence. Therefore, only the control verse saracatinib treatment groups will be discussed here. We will repeat this experimental set-up but use metformin instead as a more reliable autophagy modulator. Tumor samples were collected at days 0, 30 and 150 (as summarized in the flow chart, Figure 9), total RNA was extracted for cDNA microarray analysis using Affymetrix Human Genome U133 Plus 2.0 chips. Heat maps (Figure 10) were generated and pathway analysis of gene differentially expressed in 150d control and saracatinib tumor samples was performed based on the gene profile. To give a couple of examples, the first heatmap showed averaged Cx-150d+AZD samples compared to averaged Cx-150Ctl ones: 2,582 genes differentially expressed 1,436 genes up-regulated 1,146 genes down-regulated. The saracatinib-response signature was determined by defining the common set of genes that were differentially-regulated by saracatinib in 2 different studies: CWR22 and A549. This signature of 520 probe sets was then used to perform unsupervised hierarchical clustering. The results suggest

that a prominent component of the set of genes that were androgen-responsive and re-instated in the Cx-150d tumors were co-regulated by Src signaling shown in the second heatmap. The pathway analysis also showed that AR,

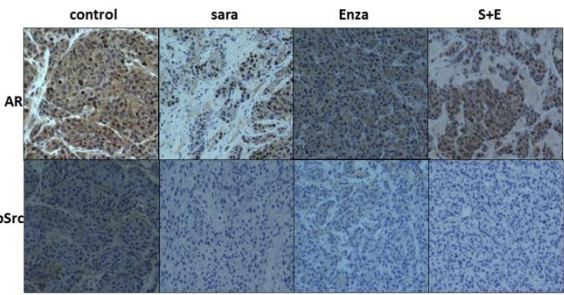


Figure 8. Tumor samples from each treatment groups were section and immunostained with anti-AR and anti-pSrc, respectively. Representative views are shown here.

After tumor volume reached 100-150 mm³, mice were surgically castrated and divided into groups, subjected to no treatment, treatments with saracatinib (25 mg/kg) or combined with chloroquine (50 mg/kg) from 7 days after surgery until 150 post-operation. CRPC recurrence was significantly inhibited with the administration of saracatinib. Mean of tumor recurrence (in tumor weight) for saracatinib, 0.18 grams and for control, 3.20 grams (p=0.015). Mean of serum PSA in the saracatinib-treatment group, 1.6 ng/ml and control, 14.7 ng/ml (P=0.08). The combination of saracatinib and chloroquine

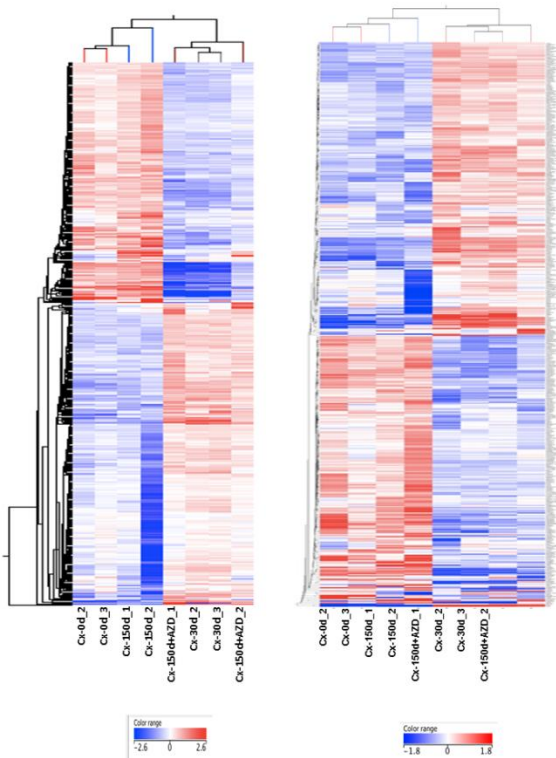


Figure 10. Two representative heatmaps generated from the gene profile for 150d control verses saracatinib (left) and saracatinib treated CWR22 xenograft verses A549 cells (right).

Table 1: Rank of changes in RNA expressions in CWR22 tumors from time points d30, d150Ctl and d150AZD with mean fold-change values represent the comparisons against d0 post-castration.

Gene Symbol	Fold change[[D30] vs [D0]]	Regulation[[D30] vs [D0]]	Fold change[[D150_CTL] vs [D0]]	Regulation[[D150_CTL] vs [D0]]	Fold change[[D150_AZD] vs [D0]]	Regulation[[D150_AZD] vs [D0]]
CORIN	33.75	up	2.58	up	40.52	up
UGT1A1,10						
etc.	25.64	down	2.68	down	11.60	down
BIRC5	24.83	down	1.12	up	9.67	down
CDK2	16.41	down	1.16	up	8.05	down
LPL	12.67	down	5.88	down	7.94	down
CCNB1	12.41	down	1.14	up	5.33	down
AKAP12	12.20	down	1.22	down	12.58	down
PTTG1	11.63	down	1.05	up	7.23	down
BCHE	10.06	up	5.52	up	9.54	up
CDC6	9.83	down	1.60	up	5.24	down
ABCA1	9.81	up	1.85	up	6.35	up
BUB1B	9.31	down	1.08	up	6.09	down
MAD2L1	8.10	down	1.10	up	5.02	down
UBE2C	8.05	down	1.12	up	5.02	down
CXCR7	7.75	up	2.18	up	8.91	up
IQGAP2	7.25	down	1.02	up	5.13	down
MYBP1	7.18	down	1.28	down	4.75	down
CDT1	6.48	down	1.18	up	4.63	down
MMP7	6.30	down	3.21	down	7.23	down
CDC45L	5.89	down	1.51	up	2.99	down
DDC	5.46	down	1.17	up	5.03	down
RACGAP1	4.86	down	1.15	up	3.46	down
ANKA3	4.65	up	1.44	up	5.68	up
IGFBP3	4.64	up	2.28	up	2.52	up
MCM4	4.52	down	1.45	up	2.59	down
CCNA2	4.44	down	1.27	up	3.23	down
PK1	4.23	down	1.34	down	6.13	down
NR3C1	4.04	up	1.01	down	3.76	up
BRCA1	4.02	down	1.35	up	2.89	down
CEBPB	3.97	down	1.22	up	1.16	down

Table 2: Changes in RNA expressions of steroid biosynthesis/AR-regulated genes in CWR22 tumors from time points d0, d30, d150Ctl and d150AZD. Mean fold-change values represent the comparisons against d0 post-castration.

Gene Symbol	Fold change[[D30] vs [D0]]	Regulation[[D30] vs [D0]]	Fold change[[D150_CTL] vs [D0]]	Regulation[[D150_CTL] vs [D0]]	Fold change[[D150_AZD] vs [D0]]	Regulation[[D150_AZD] vs [D0]]
FASN	2.78	down	1.05	down	2.43	down
CYP11A1	1.01	down	1.02	up	1.12	up
CYP17A1	1.03	down	1.15	up	1.11	up
HSD3B2	1.08	up	1.02	down	1.08	down
HSD17B2	1.04	down	1.08	up	1.06	up
HSD17B3	1.56	up	1.10	up	1.66	up
HSD17B4	1.47	down	1.06	down	1.33	down
HSD17B10	1.47	down	1.16	up	1.42	down
AKR1C1	2.32	up	1.11	up	1.05	up
AKR1C2	1.91	up	1.11	up	1.07	up
AKR1C3	1.05	up	1.12	down	1.45	down
RDH5	1.11	down	1.16	up	1.17	down
SRD5A1	1.11	down	1.19	down	1.22	down
SRD5A2	1.11	up	1.04	up	1.10	up
SRD5A3	2.45	down	1.40	down	2.51	down
UGT2B15	18.79	up	2.21	up	12.98	up
UGT2B17	23.50	up	1.51	up	17.67	up
AMACR ///						
C10TNF3	1.44	down	1.30	down	1.26	down
CYB5A	1.22	down	1.36	up	1.22	down
AR	2.41	up	2.21	up	1.69	up
EGR1	1.22	down	1.00	up	1.89	down
KLK3	1.04	down	1.10	down	1.09	up
TMPS2	1.21	down	1.28	down	1.04	down

CXCR4, MMP1 and a whole array of cell cycle genes such as CDC2, CDC20, CHEK1, RAD51 etc. may be the nodes in connecting pathways among molecules responding to castration, recurrence or Src kinase inhibition. From the microarray data, three groups of genes were tabulated and compared their response in the course of castration and treatments. The line-up of genes with the most change, either up or down-regulated was shown in the first table (Table 1). Steroid biosynthetic enzymes and AR regulated genes were grouped together (Table 2) and the third table showed genes involved in kinase activation. RT qPCR analysis was performed for

selected genes of interest to validate the microarray data (data not shown).

Table 3: Changes in RNA expressions of pathway genes in CWR22 tumors from time points d0, d30, d150Ctl and d150AZD. Mean fold-change values represent the comparisons against d0 post-castration.

Gene Symbol	Fold change[[D30] vs [D0]]	Regulation[[D30] vs [D0]]	Fold change[[D150_CTL] vs [D0]]	Regulation[[D150_CTL] vs [D0]]	Fold change[[D150_AZD] vs [D0]]	Regulation[[D150_AZD] vs [D0]]
MYC	1.08	down	1.18	up	1.20	down
SRC	1.35	down	1.05	up	1.44	down
BMX	1.02	up	1.65	down	1.88	down
PTK2	1.77	down	1.14	up	1.36	down
CTNFB1	1.66	up	1.01	up	1.45	up
JAK1	2.25	up	1.21	up	2.26	up
JAK2	1.81	up	1.16	up	1.56	up
STAT3	1.10	up	1.10	down	1.10	up
NFKB1	1.04	down	1.18	up	1.14	up
NFKB2	1.27	up	1.14	up	1.15	up
AKT1	1.28	down	1.10	up	1.07	down
AKT2	1.03	down	1.16	up	1.08	down
AKT3	1.00	down	1.21	up	1.04	down
FRAP1	3.14	down	1.05	down	2.32	down
AURKA	9.73	down	1.03	down	5.35	down
AURKB	6.36	down	1.13	up	4.95	down

Key Research Accomplishments:

1. Compiled an up and down regulation gene profile in the Enzalutamide-resistant cell and the trend was validated by qPCR in both cell and tumor background.
2. In vivo studies with CRPC cell lines demonstrated proof of principle for the role of autophagy in anti-androgen therapies combined use with two autophagy modulators, CMI and metformin.
3. Demonstrated the effects of combined use of anti-androgen and kinase inhibitor on the CRPC LNCaP-GRP in vivo model, target therapy may mechanistically block both neuropeptide and intracrine androgen mediated AR signaling axes.
4. A preliminary in vivo study on molecules behind castration resistance using the CWR22 xenograft model.

Conclusion:

In year 2, we extended our studies to in vivo animal experiments. Three animal models, LNCaP C4-2B MDV-R, LNCaP GRP Pro cells and CWR22 xenograft were used. In LNCaP C4-2B MDV-R study, we validated that using autophagy modulators such as clomipramine and metformin, tumor survival mechanism encountering enzalutamide treatment may be blocked. A much significant reduction of tumor

growth (91% and 78% for combinational use of CMI and metf with enzalutamide, respectively) was observed. In the LNCaP GRP model, combinational treatment of enzalutamide and saracatinib provided the best tumor suppression. Both specific mechanisms GRP cells utilizes for aberrant AR activation, intracrine androgen synthesis and kinase pathway mediated AR activation in the absence of androgen, were prevented through combined target therapy. Lastly, castration resistant gene activation and tumor recurrence was inhibited by saracatinib treatment in the CWR22 xenograft model but could be further enhanced when combined with autophagy modulator like metformin.

Publications, Abstracts, and Presentations:

Poster presentation:

Targeting Autophagy Overcomes Enzalutamide Resistance in Castrate Resistant Prostate Cancer Cells and Improves Therapeutic Response in a Xenograft Model HG Nguyen, JC Yang, H-J Kung, XB Shi, D Tilki, RW deVere White, AC Gao and CP Evans AACR 2014

Manuscript:

Targeting Autophagy Overcomes Enzalutamide Resistance in Castrate Resistant Prostate Cancer Cells and Improves Therapeutic Response in a Xenograft Model, HG Nguyen, JC Yang, HJ Kung, XB Shi, D Tilki, RW deVere White, AC Gao and CP Evans. *Oncogene* 2014. **33**(36): 4521-30.

Inventions, Patents and Licenses: N/A

Reportable Outcomes: None

Other Achievements: None

References:

1. Chhipa, R.R., Y. Wu, and C. Ip, *AMPK-mediated autophagy is a survival mechanism in androgen-dependent prostate cancer cells subjected to androgen deprivation and hypoxia*. Cell Signal, 2011. **23**(9): p. 1466-72.
2. Gwinn, D.M., et al., *AMPK phosphorylation of raptor mediates a metabolic checkpoint*. Mol Cell, 2008. **30**(2): p. 214-26.
3. Kim, J., et al., *AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1*. Nat Cell Biol, 2011. **13**(2): p. 132-41.
4. Nguyen, H.G., et al., *Targeting autophagy overcomes Enzalutamide resistance in castration-resistant prostate cancer cells and improves therapeutic response in a xenograft model*. Oncogene, 2014. **33**(36): p. 4521-30.
5. Wu, Z., et al., *Autophagy Blockade Sensitizes Prostate Cancer Cells towards Src Family Kinase Inhibitors*. Genes Cancer, 2010. **1**(1): p. 40-9.
6. Gillman, P.K., *Tricyclic antidepressant pharmacology and therapeutic drug interactions updated*. Br J Pharmacol, 2007. **151**(6): p. 737-48.
7. Rossi, M., et al., *Desmethylclomipramine induces the accumulation of autophagy markers by blocking autophagic flux*. J Cell Sci, 2009. **122**(Pt 18): p. 3330-9.
8. Ben Sahra, I., et al., *Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells*. Cancer Res, 2010. **70**(6): p. 2465-75.
9. Ben Sahra, I., et al., *Metformin in cancer therapy: a new perspective for an old antidiabetic drug?* Mol Cancer Ther, 2010. **9**(5): p. 1092-9.
10. Ben Sahra, I., J.F. Tanti, and F. Bost, *The combination of metformin and 2-deoxyglucose inhibits autophagy and induces AMPK-dependent apoptosis in prostate cancer cells*. Autophagy, 2010. **6**(5): p. 670-1.

11. Selvakumaran, M., et al., *Autophagy inhibition sensitizes colon cancer cells to antiangiogenic and cytotoxic therapy*. Clin Cancer Res, 2013. **19**(11): p. 2995-3007.
12. Yang, J.C., et al., *Aberrant activation of androgen receptor in a new neuropeptide-autocrine model of androgen-insensitive prostate cancer*. Cancer Res, 2009. **69**(1): p. 151-60.
13. Chun, J.Y., et al., *Interleukin-6 regulates androgen synthesis in prostate cancer cells*. Clin Cancer Res, 2009. **15**(15): p. 4815-22.
14. Nagabhushan, M., et al., *CWR22: the first human prostate cancer xenograft with strongly androgen-dependent and relapsed strains both in vivo and in soft agar*. Cancer Res, 1996. **56**(13): p. 3042-6.
15. Pretlow, T.G., et al., *Xenografts of primary human prostatic carcinoma*. J Natl Cancer Inst, 1993. **85**(5): p. 394-8.

Appendices: None