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PRINCIPAL INVESTIGATOR: Mohammad Alyamani

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Mohammad Alyamani

E-Mail: Alyamam@ccf.org

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CLEVELAND CLINIC FOUNDATION,
THE CLEVELAND CLINIC HEALTH SYSTEM
9500 EUCLID AVE
CLEVELAND OH 44195-0001

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14. ABSTRACT

First-line treatment for advanced (metastatic) prostate cancer (PCa) is androgen deprivation therapy (ADT), either by surgical or medical castration. In many cases the cancer becomes resistant, and castration resistant prostate cancer (CRPC) develops. Abiraterone, given orally as the prodrug abiraterone acetate, is used to treat CRPC and now is used as an upfront treatment in patients with castration-sensitive prostate cancer (CSPC). Abiraterone treatment improves overall survival; however, drug resistance eventually occurs, and patients die. In our previous studies, we found that abiraterone is metabolized in patients to 7 steroidal metabolites and the first step is dependent on the 3β-hydroxysteroid dehydrogenase (3βHSD) enzyme which is encoded by *HSD3B1* gene. In vitro and in vivo studies showed that abiraterone metabolites had opposing activities toward prostate tumor cells. Overall this project aims to investigate the steroidogenic metabolism of abiraterone and identify biomarkers of resistance. Here, we studied the pharmacokinetics of abiraterone metabolites after a single dose of abiraterone acetate in healthy subjects and used the data to normalize the levels of the metabolites in CSPC patients treated with abiraterone acetate to evaluate the association between abiraterone metabolites and the status of the *HSD3B1*. My results suggest no association between *HSD3B1* genotype and the formation of abiraterone metabolites. In prostate cancer cell lines, I found that abiraterone metabolites will shift the metabolism of endogenous steroids and also mediate the expression of the androgen receptor-regulated genes. These data suggest that abiraterone metabolites may serve as indirect cause of treatment resistance in CSPC patients treated with abiraterone acetate.

15. SUBJECT TERMS
Abiraterone, Biomarkers, Castration sensitive prostate cancer

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1. INTRODUCTION:

Prostate cancer (PCa) is a major health problem in the United States, being the most frequently diagnosed cancer and the second leading cause of cancer-related death in men. Abiraterone, giving as the pro drug abiraterone acetate, is a potent steroidal inhibitor of CYP17A1 which is approved to treat PCa patients. We have reported that in humans, abiraterone is metabolized by steroidogenic enzymes to at least 7 steroidal compounds. Further, *in vitro* and *in vivo* these abiraterone metabolites exert opposing effects with respect to prostate cancer progression. These findings suggest that abiraterone metabolism generates compounds that prevent CRPC progression and others that can cause treatment resistance in CRPC. Therefore, I hypothesize that abiraterone steroidal metabolites play a crucial role in the development of treatment resistance in CRPC and can serve as biomarkers that will predict resistance to abiraterone treatment in patients with metastatic castration-sensitive prostate cancer. In this project I will identify and confirm the identified abiraterone metabolites in patients with metastatic castration-sensitive PCa and determine the relationship between the metabolite levels and clinical outcomes, and then determine mechanistically whether the abiraterone metabolites are a direct or indirect cause of abiraterone resistance.

2. KEYWORDS:

Abiraterone, Biomarkers, Castration sensitive prostate cancer, *HSD3B1*

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Training-Specific Tasks:

Major Task 1: Training and educational development in prostate cancer research

Milestone(s) Achieved: Completion of Research Conduct and Human Subjects Training. (24 Months)

Presentation of project data at national meetings. (24 Months)

Research-Specific Tasks:

Specific Aim 1: Identify and confirm the identified abiraterone metabolites in patients with metastatic castration-sensitive PCa and determine the relationship between the metabolite levels and clinical outcomes.

Major Task 1: Generate a data set from an available pharmacokinetic study of abiraterone acetate.

Milestone(s) Achieved: Generating the reference value for each of the 7 steroidal metabolites to be used to compare with the metabolites values in the patients. (3 Months)

Major Task 2: Normalize the metabolite levels in patients.

Milestone(s) Achieved: generating the normalized value that will be correlate with the clinical outcomes. (12 Months)

Major Task 3: Correlate the metabolite levels with clinical outcomes.

Milestone(s) Achieved: Determination of correlation between the metabolites and the clinical outcome of each patient to define biomarkers for drug resistance. (12 Months)

Specific Aim 2. Determine mechanistically whether the abiraterone metabolites are a direct or indirect cause of abiraterone resistance.

Major Task 4: Determine the effect of abiraterone metabolites on steroidogenic enzymes *in vitro.*

*Milestone(s) Achieved: Determination of the effect of the metabolites on steroidogenic enzyme activity. (*20 Months)

Major Task 5: Determine whether abiraterone metabolites mediate AR-regulated gene expression *in vitro.*

*Milestone(s) Achieved: Determination of the effect of the metabolites on AR regulated gene expression; publication in peer review journals. (*24 Months)

What was accomplished under these goals?

Specific Aim 1: Identify and confirm the identified abiraterone metabolites in patients with metastatic castration-sensitive PCa and determine the relationship between the metabolite levels and clinical outcomes.

Major Task 1: Generate a data set from an available pharmacokinetic study of abiraterone acetate.

Milestone(s) Achieved: Generating the reference value for each of the 7 steroidal metabolites to be used to compare with the metabolites values in the patients. (3 Months)

Results: To correct for variations between the last AA dose and blood draw among individual patients with PCa, the abiraterone metabolite concentrations (D4A, 3-keto-5α-Abi, 3α-OH-5α-Abi, 3β-OH-5α-Abi, 3-keto-5β-Abi, 3α-OH-5β-Abi, and 3β-OH-5β-Abi) in the patients will be normalized to a pharmacokinetic PK study in healthy controls to account for their respective PK parameters. Samples from 15 healthy male volunteer were analyzed by LC-MS. In the PK study, the volunteers received a single dose of AA, 1000 mg, plasma samples were collected at -0.5 (pre-dose), 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours, and analyzed for steroidal abiraterone metabolites by LC-MS/MS method. The results from this study were used to generate reference data for abiraterone and the metabolites. **(Annual report "Table 1 and Figure 1")**

Major Task 2: Normalize the metabolite levels in patients.

Milestone(s) Achieved: generating the normalized value that will be correlate with the clinical outcomes. (12 Months)

Results: We received the Approval from the Human Research Protection Office (HRPO) to perform this study. However due to COVID-19 we were not able to recruit 80 patients. 65 patients consented to the study and only 38 had started the treatment and had samples available for LC-MS analysis.

Major Task 3: Correlate the metabolite levels with clinical outcomes.

Milestone(s) Achieved: Determination of correlation between the metabolites and the clinical outcome of each patient to define biomarkers for drug resistance. (12 Months)

Results: We planned to correlate the results with clinical outcomes as listed in sub task 1 of major task 3 we extracted the genotype from these patients, we found that n= 15 patients are homozygous wild type, n=22 are heterozygous, and n=1 homozygous variant *HSD3B1* genotype. Since we only had one patients with the homozygous variant we could not compare the three genotypes. We only compared two genotype the homozygous wild type and the heterozygous variant and correlates the levels of the metabolites to the genotype **Table.1 & Figure 1**. The results show no association between *HSD3B1* genotype and abiraterone metabolites levels. In this cohort the differences in levels of abiraterone metabolites among *HSD3B1* genotypes did not reach statistical significance **Figure 1**.

Specific Aim 2. Determine mechanistically whether the abiraterone metabolites are a direct or indirect cause of abiraterone resistance.

Major Task 4: Determine the effect of abiraterone metabolites on steroidogenic enzymes *in vitro.*

*Milestone(s) Achieved: Determination of the effect of the metabolites on steroidogenic enzyme activity. (*20 Months)

Results: To study the effect of abiraterone metabolites on steroidogenic enzyme activity, $1*10^6$ cells of the prostate cancer cell lines LnCaP, LAPC4, and C4-2 were used. Media and cell pellets were subject to LC-MS analysis to determine the levels of androgens as well as the effect of abiraterone metabolites on the levels of the intended androgen. In LnCaP cells, 1µM of Dehydroepiandrosterone DHEA, 3β- Androsterone 3β-AST, and 3β-Androstanediol 3β-diol with or without 1µM of the following abiraterone metabolites D4A, 3β-hydroxy-5α-Abi, and 3β-hydroxy-5β-Abi were incubated for up to 72 hours. Samples were collected at 24, 48, and 72 hours. Abiraterone metabolites has an effect on androgen metabolism and can shift the pathways suggesting that abiraterone metabolites plays important role in regulating steroidogenesis. The results also suggest that DHEA is still detected and metabolized up to 72 hours, however 3β-AST and 3β-diol levels were low at 24 hours and 3β-diol cannot be detected at the 48 hour time point. This can be explained by the fact that both 3β-AST and 3β-diol can be radially glucoronidated via the UGT enzyme that is presence in LnCaP cells limiting our studies in these cell lines to the 24 and 48 hour time point for 3β-diol and 3β-AST respectively. LnCaP cells results are in **(Annual report "Figures 2-4")**. For both LAPC4 and C4-2, 100nM of the androgen with or without 50nM abiraterone metabolites were incubated for up to 48 hours. Samples were collected at 4, 24, and 48 hours. For these experiments the following androgens were tested: DHEA, Testosterone T, Dihydrotestosterone DHT, 5α-Androstandione 5α-dione, 3α- Androsterone 3α-AST, 3β-AST, 3α-Androstanediol 3α-diol, and 3β-diol, in the presence of the following abiraterone metabolites, 3-keto-5α-Abi, 3keto-5β-Abi, 3α-hydroxy-5α-Abi, 3α-hydroxy-5β-Abi, 3β-hydroxy-5α-Abi, and 3β-hydroxy-5β-Abi. Each sample was repeated three technical times and each experiment was repeated three times. **Figures 2-7** are representative plots of the *In vitro* experiments. As shown in (**Figure 2)** at the 4 hour time points Abi metabolites slow the formation of 5α-dione and DHT in LAPC4 cells treated with 3α-AST and 3α-diol respectively. At 24 hours of treatment Abi metabolites had an effect only on cells treated with 3α-diol and slow DHT formation. 3α-OH-5α-Abi slows intracellular conversion of 3α-diol to DHT **Figure 2C.** In **Figure 3A** the results suggest a direct effect of tetrathydroxy Abi metabolites on T metabolism by slowing the formation of DHT in LAPC4 cells treated with T for 4 or 24 but not 48 hours. Moreover, 3-keto-5α-Abi slows media

and intracellular DHT formation in LAPC4 cells treated with T for 24 or 48 hour **Figure 3B**. Abi metabolites have no effect on DHT formation in LAPC4 cells treated with 5α-dione for 4, or 24 hours however they increased 3α-AST formation after 24 hour of 5α-dione treatment **Figure 4**. Although Abi metabolites slow the formation of T in LAPC4 cells treated with DHT they did not affect DHT levels **Figure 5**. In C4-2 cells 3β-OH-5β-Abi increased AD formation in cells treated with DHEA for 24 Hour. Both 3β-OH-5α-Abi and 3β-OH-5β-Abi increased 5α-Dione formation in cells treated with 3β-AST for 24 hour **Figure 6**. In **Figure 7** 3β-OH-5α-Abi increased the intracellular levels of DHT in C4-2 cells treated with 3β-diol for 48 hour. As shown in **Figures 2-7**, abiraterone metabolites affect androgen metabolism and can shift the pathways suggesting that abiraterone metabolites plays important role in regulating steroidogenesis.

Major Task 5: Determine whether abiraterone metabolites mediate AR-regulated gene expression *in vitro.*

*Milestone(s) Achieved: Determination of the effect of the metabolites on AR regulated gene expression; publication in peer review journals. (*24 Months)

Results: I sought to test the effect of low concentrations of 5α-Abi metabolites on AR regulated gene expression. For this purpose I used LAPC4 and C4-2 cells which are prostate cancer cell line that expresses the wild type and mutant AR respectively. Cells were serum starved for 48 hours then were treated with 3-keto-5α-Abi, 3α-hydroxy-5α-Abi, or 3β-hydroxy-5α-Abi. C4-2 cells were treated with 50, 200, and 1000 nM of the 5α-Abi metabolites for 24 and 48 hours. LAPC4 cells were treated with the following doses of 5α-Abi metabolites: 10, 50, 100, 200 nM for 48 hours. After RNA extraction and cDNA synthesis Quantitative PCR (qPCR) analysis was conducted in triplicate in an ABI 7500 Real-Time PCR machine (Applied Biosystems) using iTaq Fast SYBR Green Supermix with ROX (Bio-Rad) and primers for PSA, TMPRSS2, FKBP5 and RPLP0. In C4-2 cells at 24 hours 3-keto-5α-Abi and 3α-OH-5α-Abi induce PSA, TMPRSS2 and FKBP5 expression in dose dependent manner. However 3-keto-5α-Abi is a stronger agonist. At 48 hours all three 5α-Abi metabolites stimulates TMPRSS2 in dose dependent manner **Figure 8**. In LAPC4 none of the 5α-Abi metabolites stimulates TMPRSS2 and FKBP5 after 48 hours of treatment **Figure 9**. Only 3-keto-5α-Abi induces PSA expression at l00 and 200 nM levels but not at 10 or 50 (**Annual report "Figures 5")**. I then sought to study the metabolism of 3-keto-5α-Abi in LAPC4 to better investigate the effect of this metabolite on PSA. Same conditions were repeated concurrently with three concentrations of 3-keto-5α-Abi 10, 50, and 200 nM to evaluate 3-keto-5α-Abi metabolism in LAPC4 and media samples were collected and were subject to LC-MS analysis to determine 3-keto-5α-Abi metabolism. I found that 3-keto-5α-Abi will be converted to 3α-OH-5α-Abi and 3β-OH-5α-Abi and that the percentage left of 3-keto-5α-Abi is almost 25 % regardless of the initial concentration. (**Annual report "Figures 5-7 and Table 3")**

Table1: Metabolite concentrations (ng/ml) according to *HSD3B1* genotype in patients with castration-sensitive prostate cancer

HSD3β1 Genotype	Dose to Draw (H:Min)*	Abi	D4A	3-Keto-5α-Abi	3α-OH-5α-Abi	3β-OH-5α-Abi	3-Keto-5β-Abi	3α-OH-5β-Abi	3β-OH-5β-Abi
HZ	1:00	73.54	2.08	4.64	0.57	0.36	3.48	8.30	4.24
HZ	2:00	46.52	8.97	15.02	2.19	3.05	20.24	53.96	68.60
HZ	2:00	127.14	4.83	24.52	2.22	1.59	10.33	16.09	11.15
HZ	2:24	124.32	10.33	9.66	1.30	0.49	11.08	25.88	14.94
HZ	2:30	75.29	6.30	12.98	1.29	0.57	6.46	21.20	12.10
HZ	2:50	7.62	3.36	3.25	0.74	0.15	12.01	41.20	22.43
HZ	3:25	46.94	1.94	3.61	0.76	0.34	0.73	3.52	2.72
HZ	3:30	36.37	1.68	22.76	2.94	4.91	6.56	18.96	19.45
HZ	3:36	135.85	7.19	41.62	2.63	1.01	26.96	15.72	16.17
HZ	3:39	85.27	2.64	15.25	2.45	0.81	3.72	9.58	6.80
HZ	4:00	221.29	8.61	25.22	4.73	2.26	5.44	18.93	13.82
HZ	4:45	30.00	3.46	3.10	0.65	0.47	8.36	22.93	20.25
HZ	5:55	30.30	2.67	13.66	2.65	1.67	5.04	22.54	16.24
HZ	6:00	70.43	3.66	21.29	2.49	0.90	9.19	11.70	9.45
HZ	6:20	50.49	5.79	13.93	1.59	0.66	5.77	9.28	9.21
HZ	7:20	18.06	1.60	1.68	0.31	0.25	2.40	7.42	4.69
HZ	10:00	27.64	2.47	9.44	1.52	0.99	2.56	8.77	6.81
HZ	11:00	25.87	3.88	12.37	1.86	1.00	8.19	24.01	29.85
HZ	11:00	17.65	1.23	3.29	0.49	0.19	1.45	3.55	1.94
HZ	12:30	22.65	2.56	7.82	2.17	0.49	4.38	14.11	8.86
HZ	14:13	35.26	2.69	3.39	0.65	0.30	2.30	6.97	5.29
HZ	19:00	28.28	3.07	6.09	1.25	1.07	5.93	20.73	14.96
WT	0:10	22.45	1.99	5.10	0.94	0.37	1.83	5.30	5.07
WT	1:00	5.59	0.44	1.12	0.34	2.60	0.11	0.13	10.52
WT	1:00	13.05	3.78	4.02	1.04	0.51	9.79	51.31	34.79
WT	2:21	33.28	2.96	3.92	0.76	0.91	3.85	13.99	9.78
WT	2:28	32.40	1.02	3.33	0.52	0.22	0.94	2.53	2.82
WT	2:30	151.09	8.45	20.01	2.37	0.89	8.50	20.29	11.25
WT	2:41	25.00	2.75	5.32	0.39	0.25	4.99	3.55	2.96
WT	4:00	8.64	0.83	4.31	0.71	0.17	3.40	9.94	6.56
WT	4:20	63.59	5.32	13.19	2.59	1.47	7.24	32.79	32.59
WT	5:30	66.44	5.32	7.56	1.13	0.52	6.81	20.71	13.66
WT	6:00	72.93	5.66	17.79	3.06	1.24	7.13	26.94	24.06
WT	7:10	32.35	1.59	8.41	1.89	0.59	1.22	3.55	2.37
WT	9:00	101.09	6.62	27.68	5.00	1.95	8.52	25.74	18.93
WT	10:00	25.61	1.67	5.28	1.01	0.39	1.53	4.81	3.11
WT	12:25	18.32	1.34	7.17	1.33	0.93	5.14	13.08	11.90
MT	2:30	36.53	2.05	5.63	1.19	0.39	5.48	18.57	10.22

*Time from last AA dose to blood draw
WT = wild-type
HZ = heterozygous mutant
MT = homozygous mutant

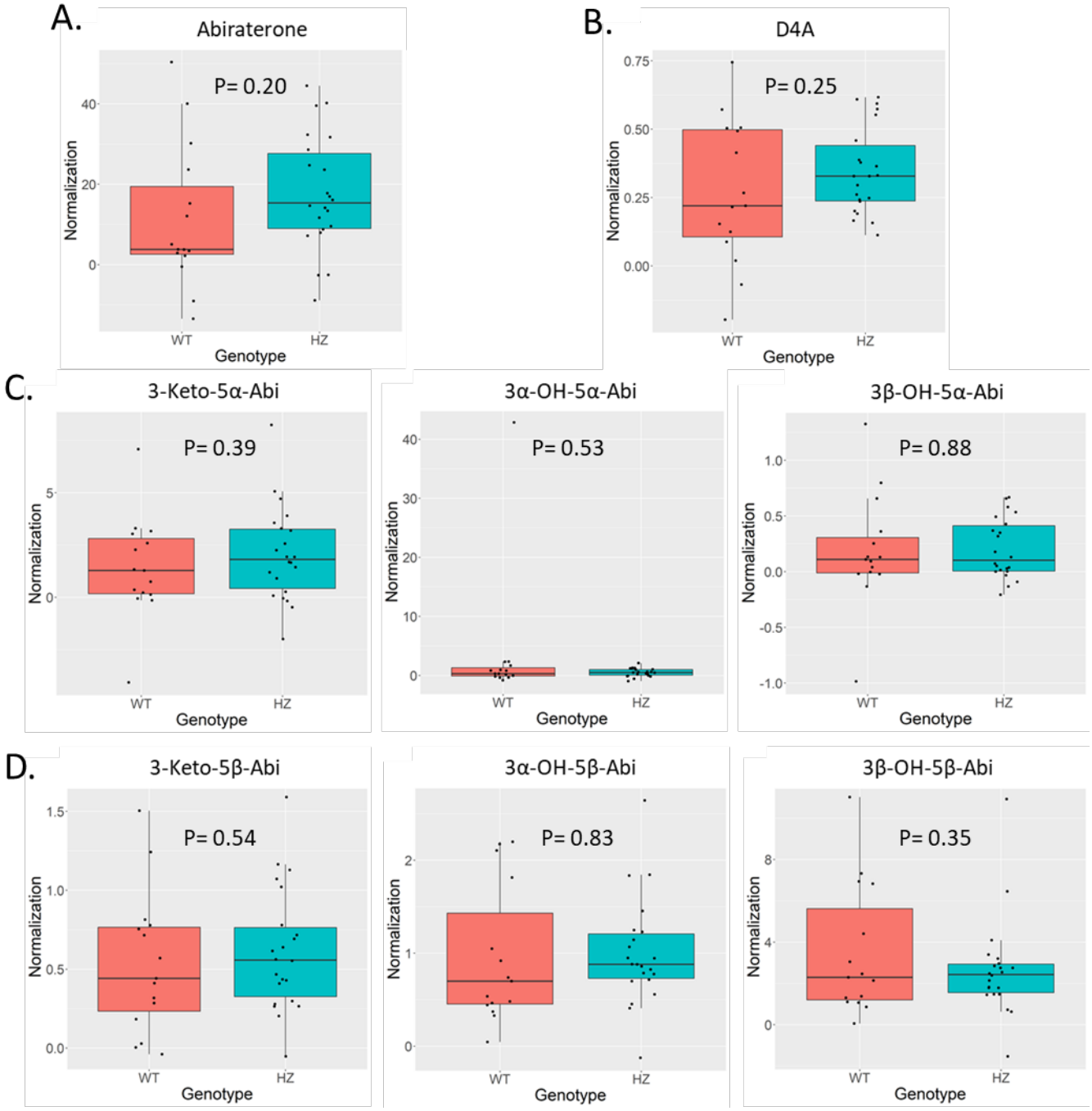

Figure 1. Association of *HSD3B1*(1245C) variant inheritance with abiraterone metabolites in 38 patients with CSPC treated with abiraterone acetate plus ADT and draw blood between 1-19 hours. WT:15, HZ:22, MT:1. A., Abiraterone, B., D4A, C., 5α-Abi metabolites, and D. 5β-Abi metabolites. The levels of each metabolite were normalized to the 8 hour value in the pharmacokinetics study and the metabolite concentration for each patient at the fixed time point is predicted by a regression method. The significance of the difference among genotypes for each metabolite was determine by polyserial correlation analysis.

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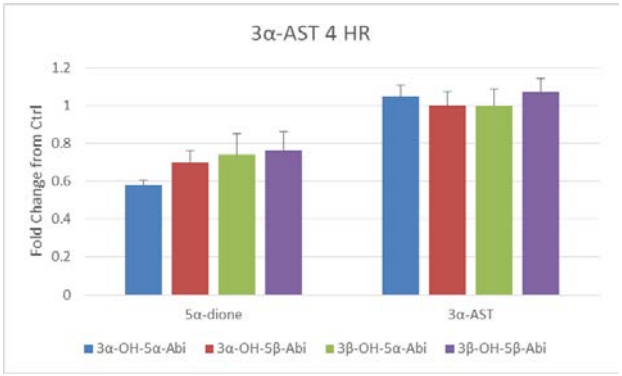

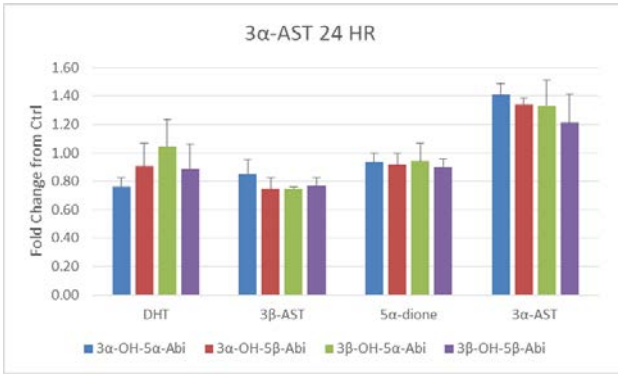

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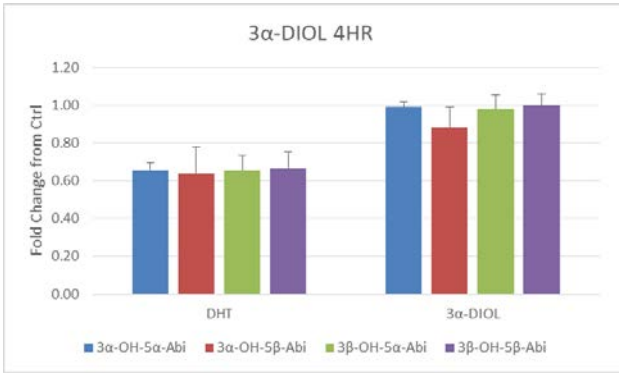

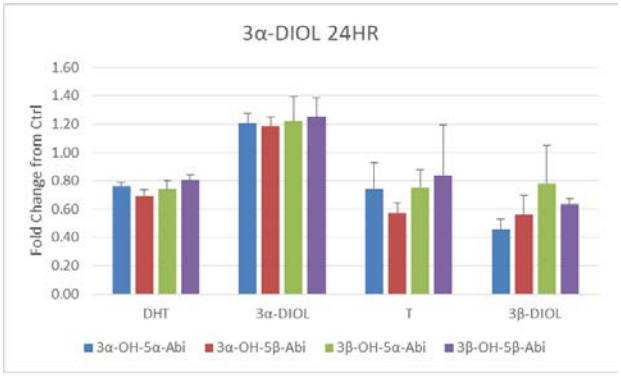

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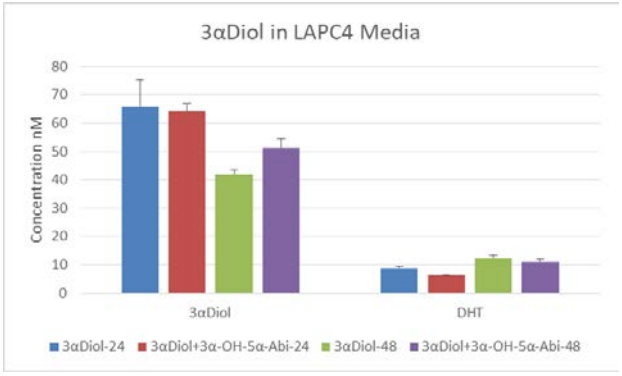
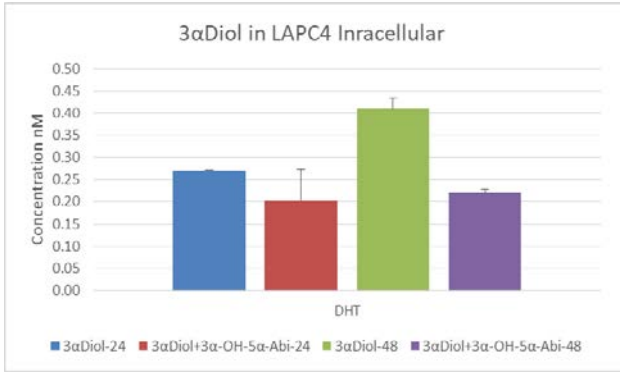
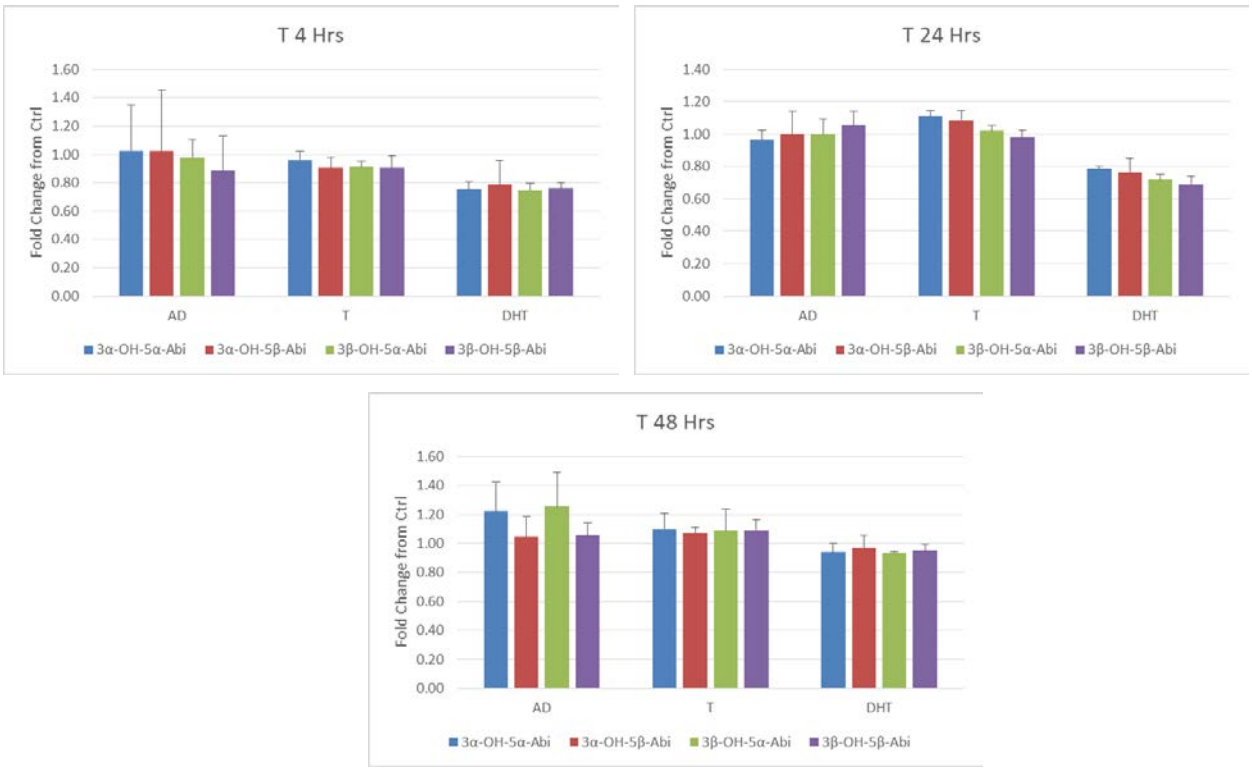




Fgure 2. Representative plots of the effect of tetraydroxy abi metabiltes on A. 3α-Androsterone (3α-AST) and B. 3α- Androstanediol (3α-diol) metabolism in LAPC4. C. The comparisone between media and intracellular metabolism of 3α-diol in the presence of 3α-hydroxy-5α-Abi. 100 nM of 3α-AST or 3α-diol was incubated in LAPC4 cells without or with 50 nM of tetrathydroxy abiraterone metabolites; 3α-hydroxy-5α-Abi, 3β-hydroxy-5α-Abi, 3α-hydroxy-5β-Abi or 3β-hydroxy-5β-Abi for 4, 24, and 48 hours. Fold change from Ctrl = (androgen + abi metabolite) treatment / ctrl “androgen alone” treatment. Error bars represent three technical repeats.

A.



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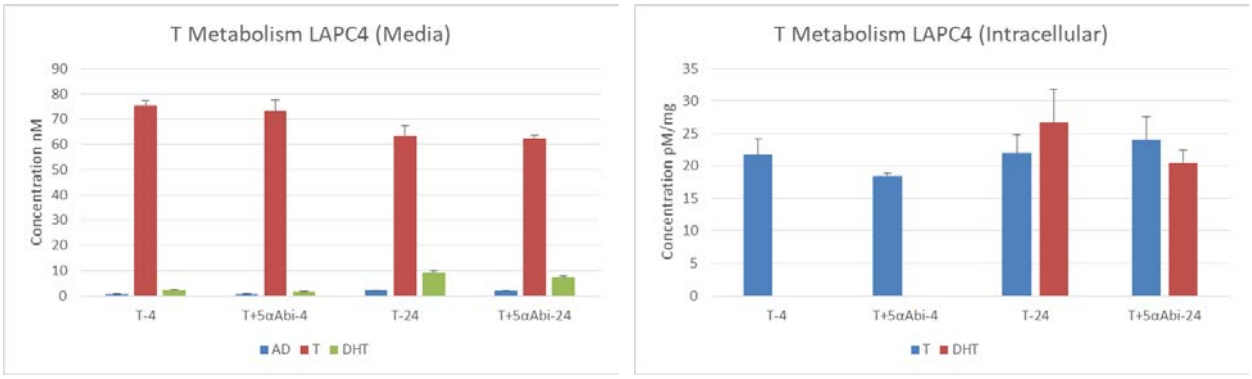
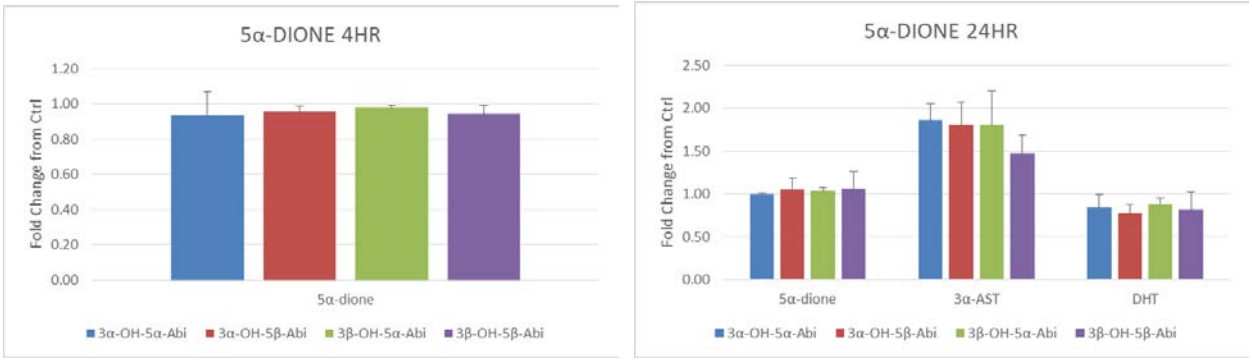


Figure 3. Representative plots of Testosterone (T) metabolism in LAPC4. A. The effect of tetraydroxy abiraterone metabiltes B. The comparisone between media and intracellular metabolism in the presence of 3-Keto-5α-Abi. 100 nM of (T) was incubated in LAPC4 cells without or with 50 nM of abiraterone metabolites for 4, 24, and 48 hours. In (A.) Fold change from Ctrl = (androgen + abi metabolite) treatment / ctrl “androgen alone” treatment. In B.) intracellular levels were normalized to cells weight. Error bars represent three technical repeats.

A.



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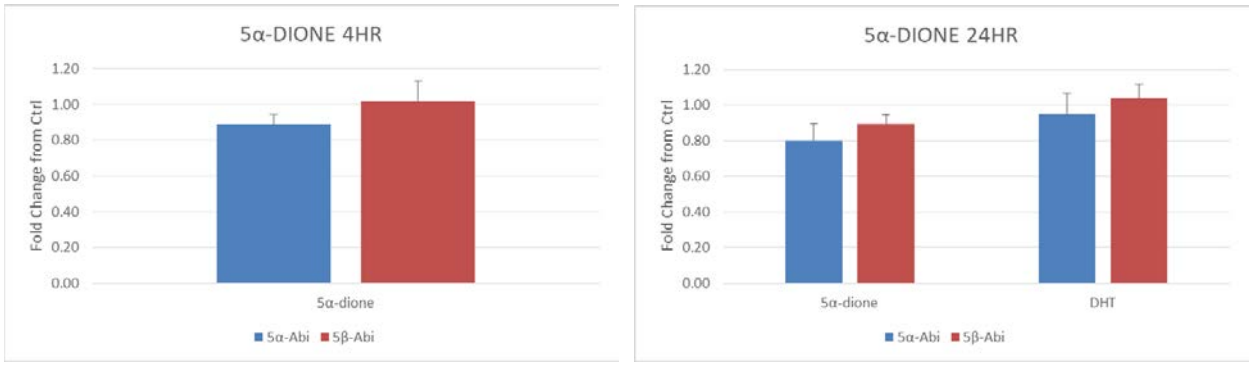
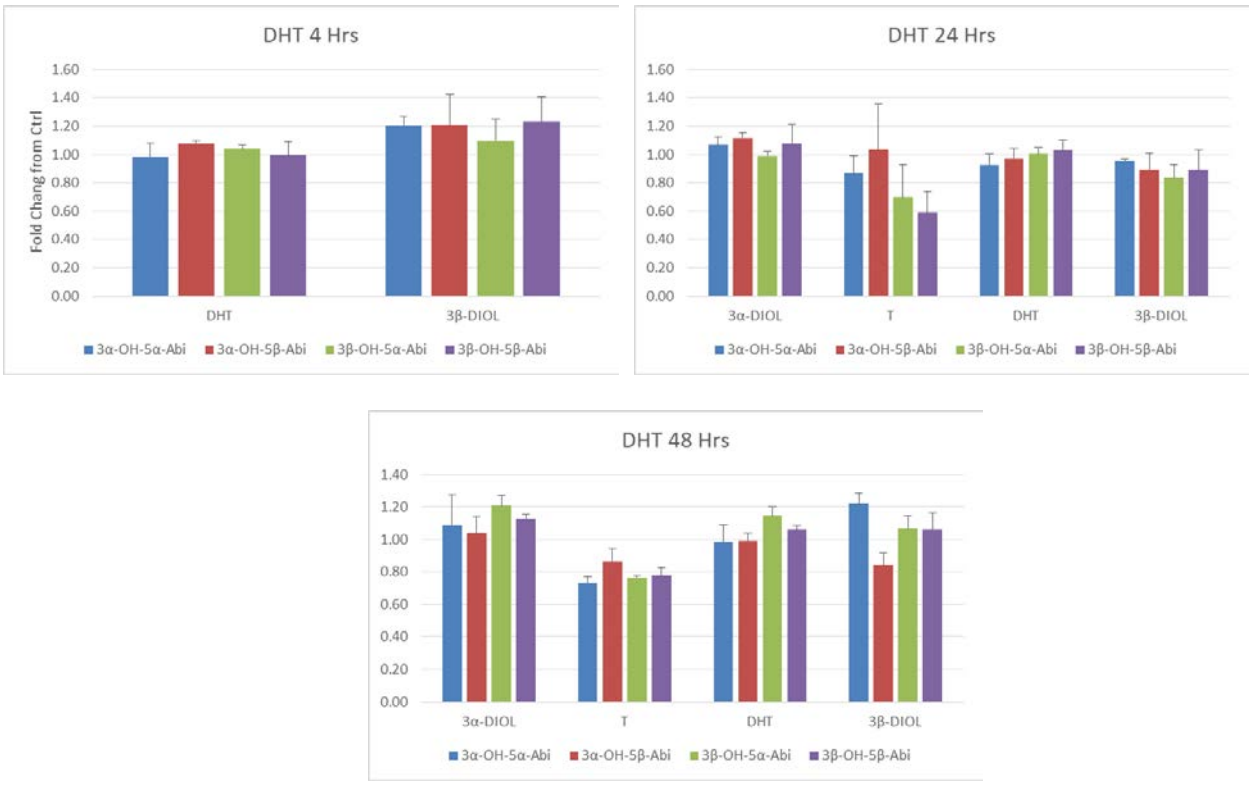


Figure 4. Representative plots of 5α-Androstandione (5α-dione) metabolism in LAPC4. A. The effect of tetraydroxy abiraterone metabiltes B. The effect of 3-keto-5α-Abi and 3-keto-5β-Abi. 100 nM of 5α-dione was incubated in LAPC4 cells without or with 50 nM of abiraterone metabolites for 4, and 24 hours. Fold change from Ctrl = (androgen + abi metabolite) treatment / ctrl “androgen alone” treatment. Error bars represent three technical repeats.

A.



B.

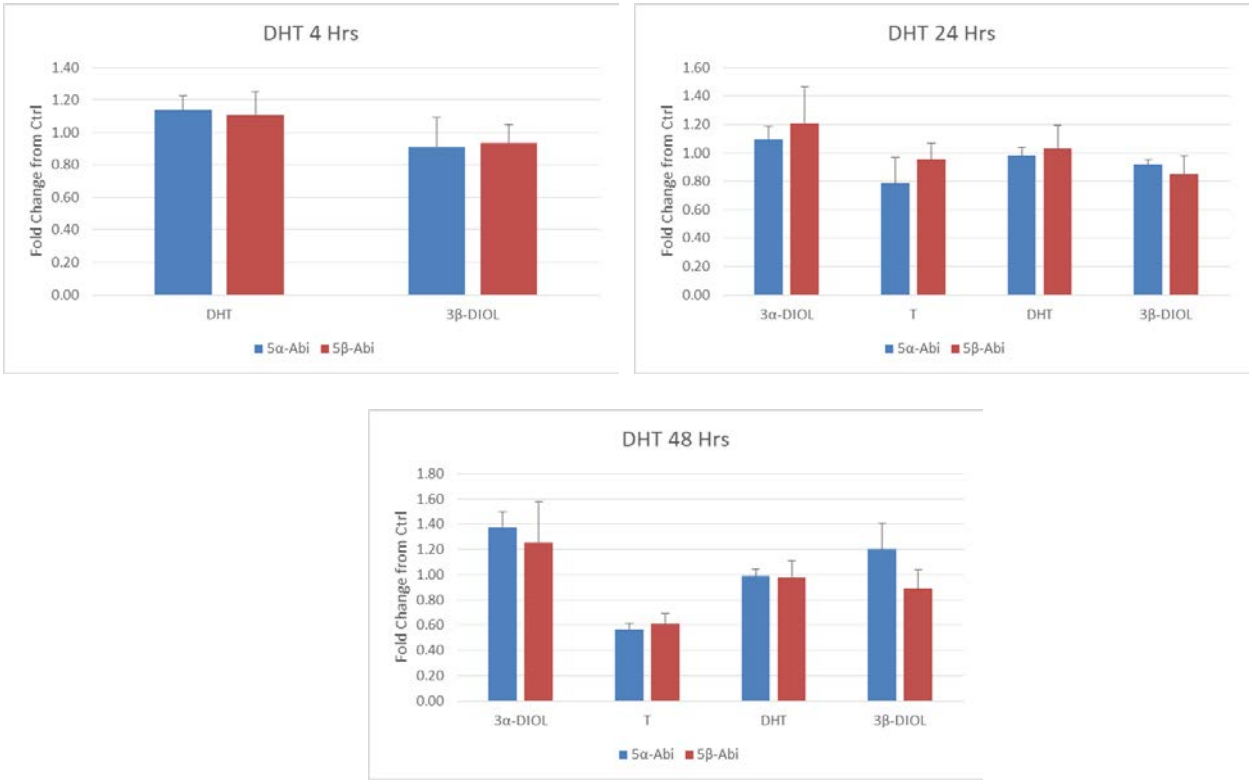
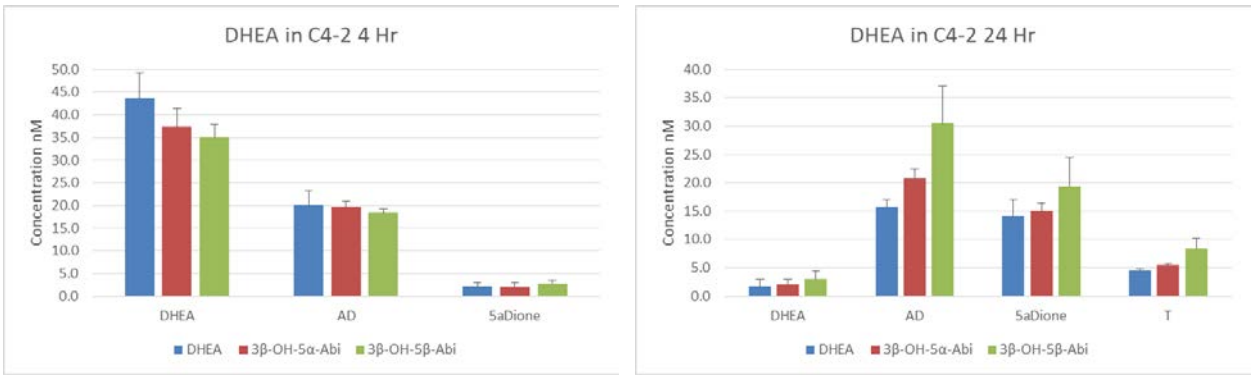


Figure 5. Representative plots of Dihydrotestosterone (DHT) metabolism in LAPC4. A. The effect of tetraydroxy abiraterone metabiltes B. The effect of 3-keto-5α-Abi and 3-keto-5β-Abi. 100 nM of DHT was incubated in LAPC4 cells without or with 50 nM of abiraterone metabolites for 4, 24, and 48 hours. Fold change from Ctrl = (androgen + abi metabolite) treatment / ctrl “androgen alone” treatment. Error bars represent three technical repeats.

A.



B.

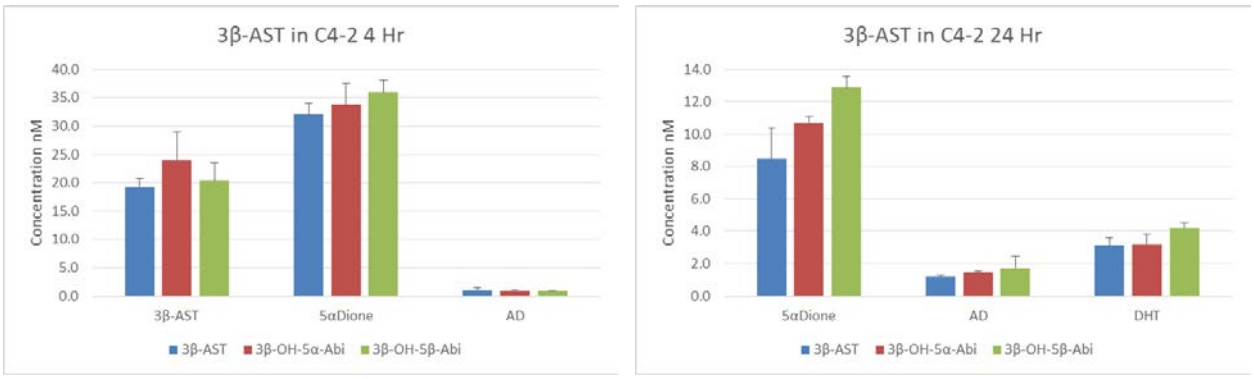


Figure 6. Representative plots of the effect of tetraydroxy abi metabiltes on A. Dehydroepiandrosterone DHEA and B. 3α-Androsterone (3α-AST) metabolism in C4-2. 100 nM of DHEA or 3α-AST was incubated in C4-2 cells without or with 50 nM of tetrathydroxy abiraterone metabolites; 3β-hydroxy-5α-Abi or 3β-hydroxy-5β-Abi for 4, or 24 hours. Concentration of the formed androgens are presented in nM. Error bars represent three technical repeats.

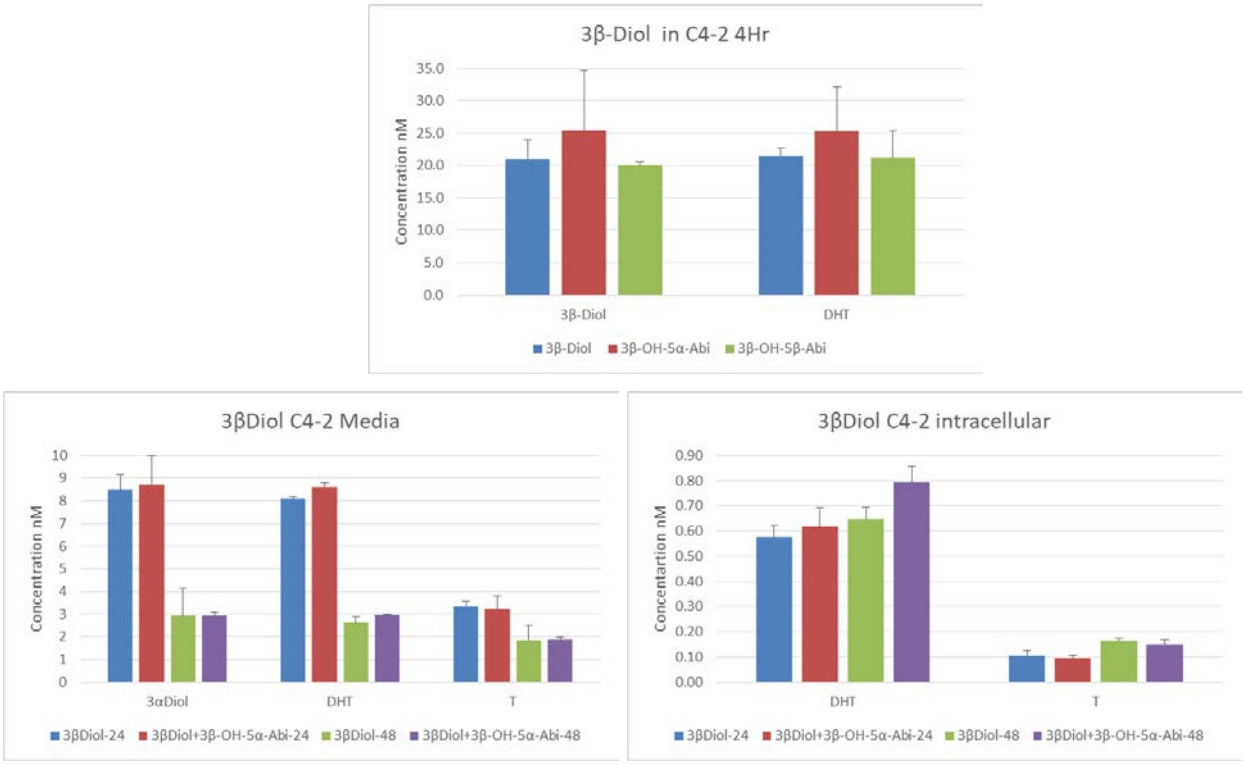
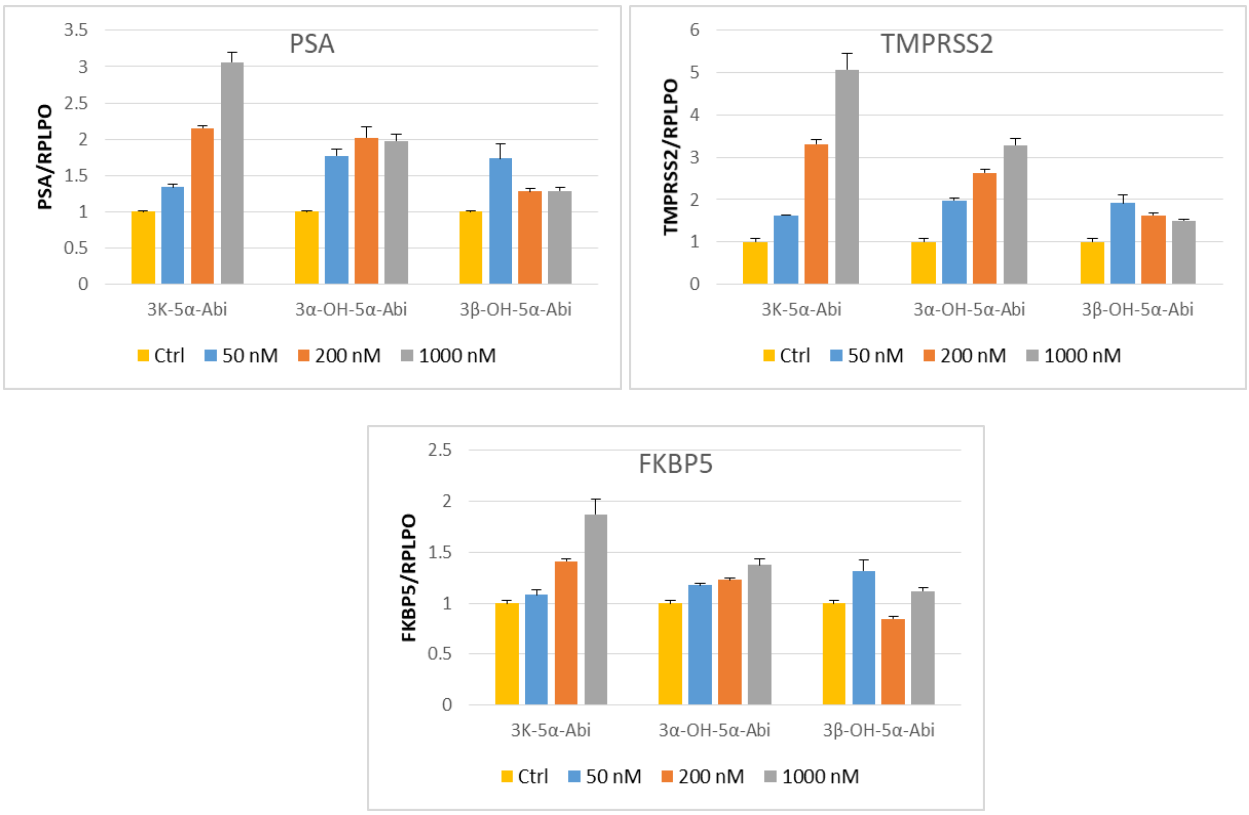


Figure 7. Representative plots of 3β- Androstanediol (3β-diol) metabolism in C4-2. A. The effect of tetraydroxy abiraterone metabiltes B. The comparisone between media and intracellular metabolism in the presence of ; 3β-hydroxy-5α-Abi. 100 nM of 3β-diol was incubated in C4-2 cells without or with 50 nM of tetrathydroxy abiraterone metabolites; 3β-hydroxy-5α-Abi or 3β-hydroxy-5β-Abi for 4, 24, and 48 hours. Concentration of the formed androgens are presented in nM Error bars represent three technical repeats.

A.



B.

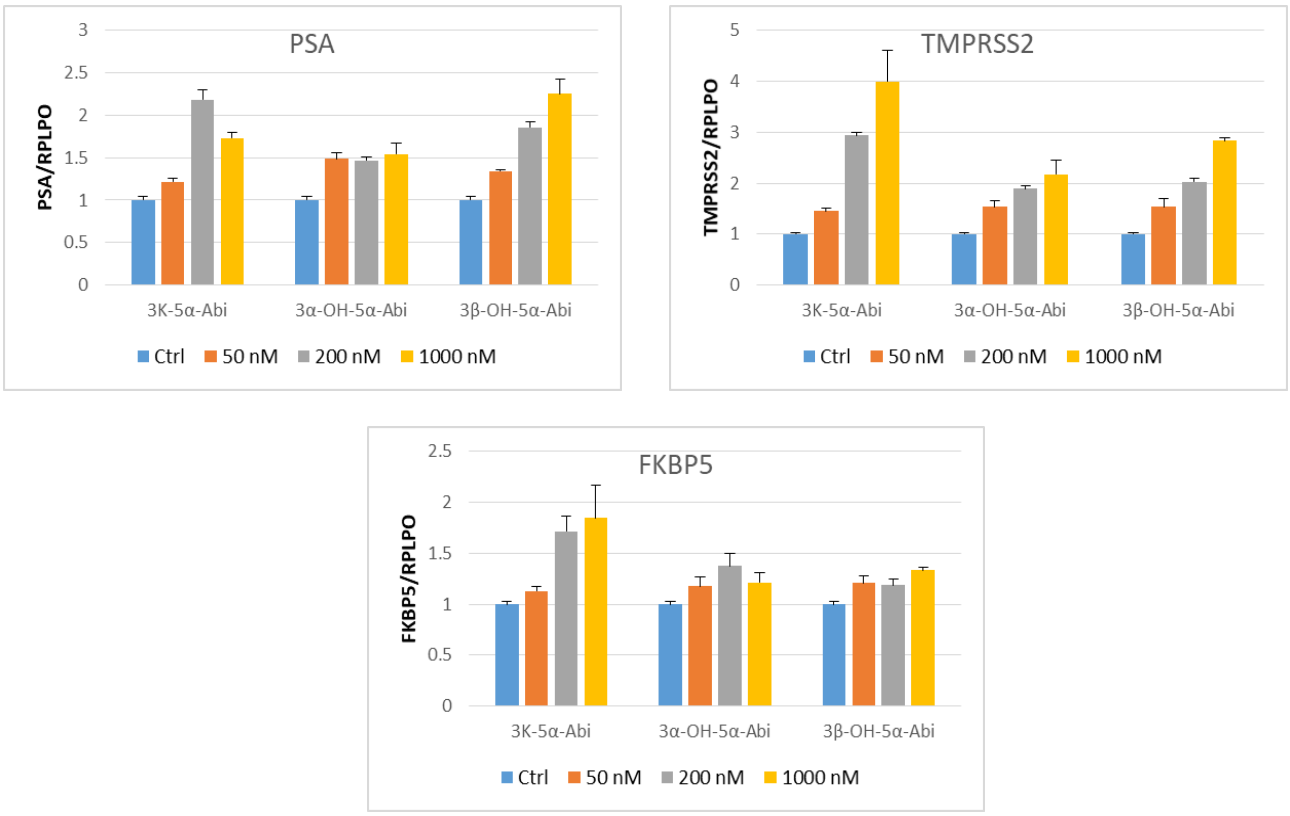


Figure 8. Effect of 5α-Abi metabolites on AR target gene expression. 10^6 C4-2 cells were serum starved for 48 houres and then treated with 50, 200 or 1000 nM of the 5α-Abi metabolites, 3-Keto-5α-Abi, 3α-hydroxy-5α-Abi, or 3β-hydroxy-5α-Abi for A. 24 and B. 48 hours. At 24 hours, 3-keto-5α-Abi and 3α-hydroxy-5α-Abi can stimulate PSA and TMPRSS2 expression when normalized to control and RPLP0. While at 48 hours 3β-hydroxy-5α-Abi is a stronger agonist than 3α-hydroxy-5α-Abi. Error bars represent SD of three technical repeats.

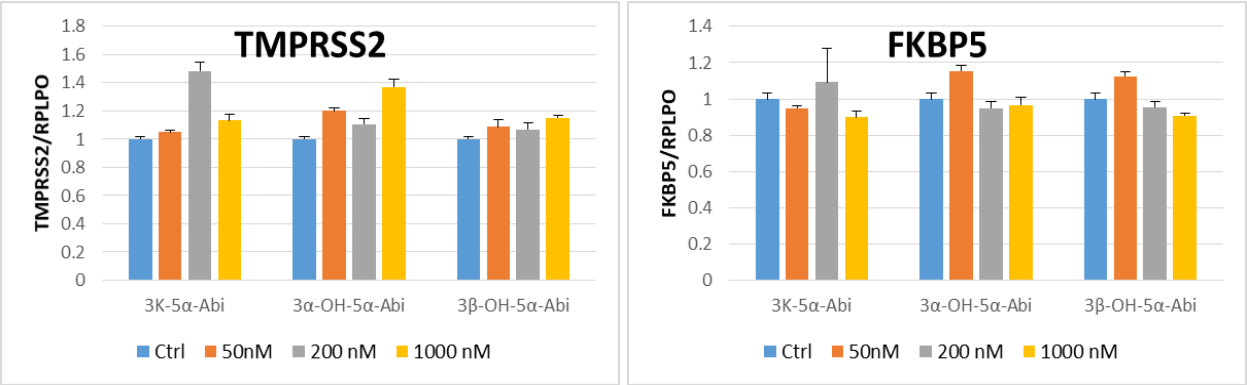


Figure 9. Effect of 5α-Abi metabolites on AR target gene expression. 10^6 LAPC4 cells were serum starved for 48 houres and then treated with 50, 200 or 1000 nM of the 5α-Abi metabolites, 3-Keto-5α-Abi, 3α-hydroxy-5α-Abi, or 3β-hydroxy-5α-Abi for 48 hours. None of the 5α-Abi metabolites had an effect on AR target genes TMPRSS2 and FKBP5 when normalized to control and RPLP0. Error bars represent SD of three technical repeats.

What opportunities for training and professional development has the project provided?

1. Attended Responsible Conduct of Research and Human Subjects Training.
2. Attended monthly Prostate Cancer Working Group and Seminar Series.
3. Attended and present research at the weekly lab meetings and journal clubs.
4. Attended and present my work at the weekly Cleveland Clinic Department of Cancer Biology seminars.
5. Attended and present at the bi-weekly Cleveland Clinic Department of Cancer Biology journal club.
6. Attended ENDO 2019
7. Attended workshop, Pharmacokinetics for Pharmaceutical Scientists Course in University of California San Francisco
8. Attended and completed Medical Biostatistics Part I and II courses at Cleveland Clinic.
9. Attended and completed Introduction to Clinical Research course at Cleveland Clinic.
10. Attended Prostate Cancer Foundation Scientific Retreat.
11. Attended Metabolomics Association of North America 1st annual meeting.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
This project reveals that the metabolism of abiraterone varies with the stage of the disease. In patients with **castration-sensitive prostate cancer,** abiraterone metabolites did not associate with the status of *HSD3B1* genotype. These results are in contrast with our previous findings where we found that in patients with **castration-resistance prostate cancer** abiraterone metabolite formation is dependent on the status of *HSD3B1* genotype. This project also suggest that Abiraterone metabolites that were considered inactive can disrupt androgen metabolism.

What was the impact on other disciplines?
Nothing to Report.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
In this funding period we planned to analyze 80 subjects, however we were able to consent only 65 patients but only 38 started the treatment.

Changes that had a significant impact on expenditures
Nothing to Report

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

Significant changes in use or care of human subjects
Nothing to Report.

Significant changes in use or care of vertebrate animals
Nothing to Report.

Significant changes in use of biohazards and/or select agents
Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.
  Nothing to Report.

  Books or other non-periodical, one-time publications.
  Nothing to Report.

  Other publications, conference papers and presentations.
  Nothing to Report.

- **Website(s) or other Internet site(s)**
  Nothing to Report.

- **Technologies or techniques**
  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report.

- **Other Products**
  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Mohammad Alyamani
Project Role: Principle Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-7127-0820
Nearest person month worked: 24

Contribution to Project: Mohammad is responsible for design, perform and interpret experiments.
Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to Report.

QUAD CHARTS:
Nothing to Report.

9. APPENDICES:

Nothing to Report.