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14. ABSTRACT

The proposed studies are expected to (1) identify genetic variations in the genes of androgen transporters that are associated with the racial differences in prostate cancer aggressiveness; (2) identify key androgen transporters of which the expression and/or the alteration of expression in cancer relative to benign prostate tissue are associated with racial differences in prostate cancer aggressiveness. Progress in the reporting period includes: 1) Completion of genotyping for all 11 SLCO members using PCaP DNA samples; 2) Finish data processing and preliminary data analyses for genotyping, and identified SNPs that may be associated with prostate cancer characteristics; 3) Continued RNAScope analysis of SLCO transporter in prostate cancer tissue sections and discover unique cell type-specific expression of a SLCO transporter; 4) Further delineation of androgen uptake mechanism on molecular levels.

15. SUBJECT TERMS

None listed.

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1. INTRODUCTION

Compared to European American (EA) men, African American (AA) men suffer higher incidence of, and greater mortality rate from prostate cancer. Results of multiple studies indicate that prostate cancer in AA men may progress faster than prostate cancer in EA men, and thereby becomes more aggressive. This study is focused specifically on identification of genetic/biological culprits that cause more aggressive types of prostate cancer in AA men. In particular, the proposed studies are focused on the question of how differences in transporter-mediated androgen uptake may contribute to the more aggressive type of prostate cancer in AA versus EA. The proposed studies are expected to (1) identify genetic variations in the genes of androgen transporters that are associated with the racial differences in prostate cancer aggressiveness; (2) identify key androgen transporters of which the expression and/or the alteration of expression in cancer relative to benign prostate tissue are associated with racial differences in prostate cancer aggressiveness.

2. KEYWORDS

Prostate cancer, health disparity, androgen, transporter, genetic variation.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1 (months 1-18): DNA samples as well as relevant clinical and epidemiological data will be requested for 2258 cases (1130 AA and 1128 EA) from the North Carolina-Louisiana Prostate Cancer Project (PCaP). A total of 952 SNPs along with a panel of 50 ancestry informative markers (AIMs) will be used for genotyping of 11 SLCO transporters. Genotyping will be performed via the GoldenGate Assay by Illumina Bead Station System in the Genomics Core Facility at Roswell Park Cancer Institute (RPCI).

Specific Aim 2 (months 7-30) is to examine in situ expression profiles of SLCO transporters in prostate tissue and investigate associations of the expression profiles with prostate cancer aggressiveness in AA and EA. Expression of SLCO transporters at transcriptional levels will be examined first in tissue microarrays (TMAs) constructed from prostate cancer and distant benign tissues of 92 AA and 92 EA patients from the Pathology Resource Network (PRN) at Roswell Park Cancer Institute (RPCI). The predominantly expressed SLCO transporters in AA or EA, and the transporters with expression significantly altered in cancer relative to benign tissues, will be selected and expression at protein levels will be examined using immunohistochemistry (IHC) on TMAs requested from the PCaP. The data on expression will be combined with the data on disease characteristics from the PCaP to investigate associations of the expression profiles with prostate cancer aggressiveness in AA and EA.

Specific Aim 3 (months 25-36) will characterize functions of candidate SLCO transporters in androgen uptake and evaluate the biological effects on AR signaling in human prostate cancer cell lines. Based on the findings from Aim 1 and Aim 2, candidate SLCO transporters will include the transporters that are predominant in either AA or EA, show significantly altered expression between tumor and benign tissue, or harbor genetic variants that are significantly associated with prostate cancer aggressiveness. Relevant cell models will be constructed using over-expression or siRNA knock-down for functional analysis.

What was accomplished under these goals?

Aim 1. Genotyping was successfully completed. In year 2, a preliminary analysis of the genotyping data identified tag SNPs that are associated with prostate cancer characteristics. Briefly, a total of 2050 individuals (993 AA and 1057 EA) were included in the final analysis with an average call rate above 95%. For single nucleotide polymorphisms (SNPs), including tagSNPs selected from Hapmap data, potential functional SNPs

selected from the literature, and a panel of 128 ancestry informative markers (AIMs), a total of 1045 SNPs were included in the final analysis with an average call rate above 98%.

To ensure the accuracy of the results, Dr. Tang and Dr. Zhu collaborated on a series of more robust analyses. During the final analysis, three critical adjustments were made. First, instead of using the ancestry component information requested from PCaP, we calculated proportion of ancestry estimate based on the panel of 128 AIMs genotyped together with SNPs of interest in the same genotyping platform. The ancestry component information requested from the PCaP is based on a panel of 50 AIMs and is not available for all the participants. A total of 70 participants were excluded from the preliminary analysis due to missing ancestry component estimation. With our own structure analysis, we are able to generate more accurate ancestry component estimates and restore missing data for all 70 participants. The Bayesian Markov Chain Monte Carlo clustering algorithms implemented in the program STRUCTURE v2.3.1 was used to generate a quantitative value that reflects the proportion of European and African ancestry present in the genome for each participant. Overall, the ancestry components between our calculation and the values from the PCaP are highly correlated, showing a correlation coefficient of 0.98 for both African and European ancestry. This result further confirms the validity of ancestry analysis. The updated ancestry component estimates are presented below in Table 1. Second, we created an "unknown" category for participants with missing value on family history to maximize the statistical power. During preliminary analysis, we noticed that a total of 163 participants presented with missing value on family history were excluded from the analysis. Comparing participants with missing values on family history, participants answered yes, and participants answered no, there was no statistically significant difference among these three groups in either tumor characteristics including Gleason score, stage and aggressiveness, or demographic characteristics including age, race and study site. Under this circumstance, instead of imputing, we decide to create additional category as unknown for this missing values to allow inclusion of these participants in the analysis. The descriptive table 1 is updated accordingly. Last, to correct for multiple comparisons, false discovery rate (FDR) approach was used to replace conservative Bonferroni correction in the preliminary analysis.

Table 1 summarizes the updated descriptive characteristics of the study population by self-reported race/ethnicity. The self-reported race status was supported by the distribution of ancestry proportions in the study population. Comparing with EA men, AA men tended to be diagnosed at younger ages and were more likely to have prostate cancer with high aggressiveness. There was no difference in other tumor characteristics including primary and sum Gleason Grade as well as clinical stage. AA and EA men had similar rate of family history of prostate cancer, showing the majority (approximately 70%) had no family history.

Table 1. Descriptive and tumor characteristics by race/ethnicity

	African American (N=993)		European American (N=1057)		P_value*
	Mean	SD	Mean	SD	
Age at diagnosis, yrs	61.9	7.8	64.2	7.9	<0.001
European ancestry	0.098	0.160	0.975	0.086	<0.0001
African ancestry	0.902	0.160	0.025	0.086	<0.0001
	N	%	N	%	
Study site					0.416
North Carolina	436	43.9%	483	45.7%	
Louisiana	557	56.1%	574	54.3%	
Family history of prostate cancer					0.299
No	656	66.0%	732	69.2%	
Yes	253	25.5%	246	23.3%	
Unkown	84	8.5%	79	7.5%	
Primary Gleason Grade					0.082
<4	770	80.0%	858	83.0%	
≥4	193	20.0%	176	17.0%	
Summary Gleason Grade					0.147
<8	861	86.9%	936	89.0%	
≥8	130	13.1%	116	11.0%	
Stage					0.825
I&II	946	98.1%	1019	98.3%	
III&IV	18	1.9%	18	1.7%	
Aggressiveness					0.0007
Low/intermediate	750	79.0%	872	84.9%	
High	199	21.0%	155	15.1%	

* Chi square test was used for categorical variables, and student t-test was used for continuous variables if normally distributed, otherwise Kruskal-Wallis test was used.

To examine associations of genetic variations in SLCO transporters with prostate cancer characteristics, Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression model adjusting for age, study site, family history of prostate cancer and African ancestry proportion. A co-dominant (genotypic) model was primarily assumed, then a dominant model was included with consideration of small

number of homozygotes for most of tested SNPs. The analysis was performed in AA and EA separately with the following four cancer characteristic outcomes: 1. prostate cancer aggressiveness (high versus intermediate/low), which is defined using three variables described as following: (i) high aggressiveness (Gleason sum ≥ 8 or PSA > 20 ng/mL or Gleason sum ≥ 7 and clinical stage T3–T4), (ii) low aggressiveness (Gleason sum < 7 and clinical stage T1–T2 and PSA < 10 ng/mL), and (iii) intermediate aggressiveness (all other cases); 2. primary Gleason Grade (≥4 versus <4); 3. sum Gleason Grade (≥8 versus <8); and 4. clinical stage (T3/4 versus T1/2). Three P values are presented: P_trend, for genetic dose response by coding genotypes as 0, 1, 2 on the basis of the number of variant alleles; P_adj, P_trend after FDR correction for multiple comparisons; and P_interaction, Wald test of the product term between race and genotype for the differences in associations between AA and EA men. All analyses were performed using R and/or SAS 9.4 (Cary, NC). Only SNPs with corrected P_trend below the level of 0.05 were presented in Table 2. Primary Gleason grade was not included in Table 2, since no SNPs were significantly associated with primary Gleason grade.

As shown in Table 2, all significant SNPs belong to two genes, SLCO2A1 and SLCO5A1. SNPs in SLCO2A1 were associated with reduced tumor aggressiveness as well as tumor sum Gleason grade; however, the associations were primary observed in AA population, but not EA population. In contrast, SNPs in SLCO5A1 were positively associated with high clinical stage, and the associations were primarily found in EA population, but not AA population. For certain SNPs, such as rs9917636 and rs3811662 in SLCO2A1 and rs16919172 in SLCO5A1, the observed associations were significantly different between AA and EA population, showing P value for interaction

Table 2. Differential associations of single-nucleotide polymorphisms in SLCO transporters with prostate cancer characteristics between African American and European American men in the PCaP study

Gene	SNP	Chr	Genotype	European American				African American				
				# high vs low	OR (95% CI)[a]	P_trend[b]	P_adj[c]	# high vs low	OR (95% CI)[a]	P_trend[b]	P_adj[c]	P_interaction[d]
Aggressiveness High vs. Low/Intermediate												
SLCO2A1	rs9917636	3	AA	33/231	1.00	0.896		62/150	1.00	<0.001	0.03	0.004
			AG	88/417	1.52 (0.98-2.36)			93/367	0.59 (0.41-0.86)			
			GG	34/222	1.04 (0.62-1.74)			44/233	0.45 (0.29-0.71)			
			GG/GG vs. AA	122/639	1.35 (0.89-2.05)			137/600	0.54 (0.38-0.77)			
SLCO2A1	rs3811662	3	GG	150/861	1.00	0.021		160/519	1.00	0.001	0.039	0.004
			GA	5/10	3.77 (1.22-11.64)			38/210	0.58 (0.39-0.86)			
			AA	0/0				1/20	0.16 (0.02-1.2)			
			GA/AA vs. GG					39/230	0.54 (0.37-0.8)			
Sum Gleason Grade ≥8 vs. <8												
SLCO2A1	rs3811662	3	GG	114/922	1.00	0.574		108/603	1.00	0.001	0.049	0.218
			GA	2/13	1.55 (0.33-7.23)			22/235	0.52 (0.32-0.84)			
			AA	0/0				0/22				
			GA/AA vs. GG					22/257	0.47 (0.29-0.77)			
SLCO2A1	rs9874493	3	AA	84/671	1.00	0.967		94/518	1.00	0.001	0.049	0.352
			AG	30/243	1.04 (0.66-1.62)			36/294	0.66 (0.44-1)			
			GG	2/22	0.88 (0.2-3.85)			0/48				
			AG/GG vs. AA	32/265	1.02 (0.66-1.59)			36/342	0.56 (0.37-0.85)			
Stage 3/4 vs. 1/2												
SLCO5A1	rs16919172	8	AA	11/914	1.00	<0.001	0.039	15/729	1.00	0.604		0.028
			AG	7/101	6.24 (2.32-16.8)			3/199	0.78 (0.22-2.75)			
			GG	0/3				0/12				
			AG/GG vs. AA	7/104	5.98 (2.23-16.08)			3/211	0.74 (0.21-2.64)			
SLCO5A1	rs4370538	8	AA	10/901	1.00	<0.001	0.035	6/468	1.00	0.101		0.25
			AG	8/113	6.95 (2.64-18.27)			10/414	2.22 (0.77-6.36)			
			GG	0/3				2/64	2.85 (0.54-14.94)			
			AG/GG vs. AA	8/116	6.67 (2.54-17.52)			12/478	2.31 (0.83-6.39)			
SLCO5A1	rs4377973	8	GG	11/908	1.00	0.001	0.039	6/447	1.00	0.331		0.383
			GC	7/108	5.93 (2.21-15.95)			11/422	2.28 (0.81-6.44)			
			CC	0/3				1/77	1.08 (0.13-9.32)			
			GC/CC vs. GG	7/111	5.68 (2.12-15.26)			12/499	2.09 (0.75-5.77)			
SLCO5A1	rs10096246	8	AA	11/911	1.00	0.001	0.039	10/572	1.00	0.526		0.078
			AG	7/105	6.04 (2.24-16.26)			7/336	1.29 (0.48-3.47)			
			GG	0/3				1/37	1.69 (0.2-14.02)			
			AG/GG vs. AA	7/108	5.79 (2.15-15.57)			8/373	1.33 (0.51-3.45)			

[a] ORs and 95% CIs were estimated from co-dominant and dominant models adjusting for age at diagnosis, state (NC or LA), 1st degree family history of prostate cancer (yes/no), African ancestry component.

[b] P_trend was estimated for genetic dose response by coding genotypes as 0, 1, 2 according to the number of variant alleles.

[c] P_adj was generated from P-trend after correction for multiple comparison by FDR. Only SNPs with P_adj less than 0.05 either from AA or EA population were presented.

<0.05. Further investigation found that the four significant SNPs in SLCO5A1 (rs16919172, rs4370538, rs4377973, rs10096246) are in high linkage disequilibrium (LD) in EA population but not AA population based on 1000 genome data, which explains the similar associations across the SNPs. However, all identified SNPs in both SLCO2A1 and SLCO5A1 are located in introns and are not in LD with any potential functional SNPs or coding SNPs, rendering further functional analysis difficult. An exome sequencing project focusing on SLCO2A1 and SLCO5A1 is under development to further delineate the signals and identify functional genetic variants. Overall, two conclusions can be drawn from the results. First, among all 11 SLCO family members, SLCO2A1 and SLCO5A1 may play an important role in prostate cancer development. This conclusion is further supported by our findings from RT-PCR examination of SLCO expression in human prostate malignant and benign tissues, showing dominant expression of SLCO2A1 and SLCO5A1 in the prostate. Second, the role

of SLCO2A1 and SLCO5A1 in prostate cancer development differs by race, showing race-specific associations between genetic variations and aggressive cancer characteristics. The manuscript is under development. We plan to incorporate some findings from Aim2 in situ expression analysis to present a more comprehensive understanding of SLCO family and prostate cancer.

To date, the proposed tasks for Aim 1 have been accomplished by 80%. We are in the process of preparing a manuscript for publication. The initial submission and revision of the manuscript may take 3-6 months. This time is requested as part of the 1-year extension without funding (EWOF).

Aim 2. The association study in Aim 1 was critical to decision making on the selection of candidate SLCO transporters for expression level determination on the 92 AA/92 EA TMA from RPCI and the TMA from PCaP. Therefore, decisions were made only after the finalization of the association study. Based on findings in Aim 1, SLCO5A1 and SLCO2A1 were the candidates for Aim 2. Another issue to address was the localization of SLCO2A1. In Year 2, RNAScope was used to determine the expression of SLCO2A1 in prostate cancer tissue specimens. Unlike SLCO5A1, the expression of SLCO2A1 was not found in the epithelial cells or cancer cells. Instead, the expression of SLCO2A1 seemed to be in the stroma. This was an important issue to address because the tissue distribution of the transporter may determine its role in prostate cancer. Two consecutive sections of a prostate cancer tissue specimens were used for SLCO2A1 RNAScope
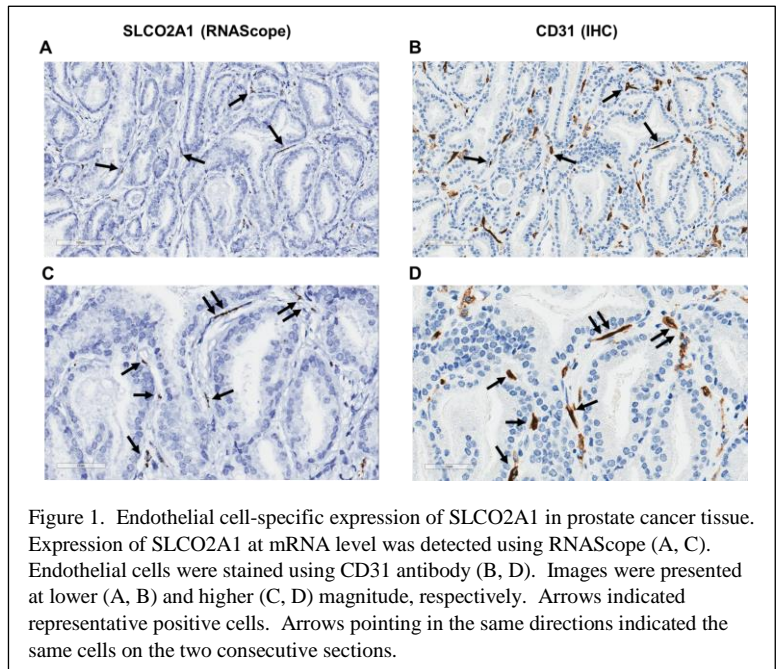

Figure 1. Endothelial cell-specific expression of SLCO2A1 in prostate cancer tissue. Expression of SLCO2A1 at mRNA level was detected using RNAScope (A, C). Endothelial cells were stained using CD31 antibody (B, D). Images were presented at lower (A, B) and higher (C, D) magnitude, respectively. Arrows indicated representative positive cells. Arrows pointing in the same directions indicated the same cells on the two consecutive sections.

and IHC staining of CD31, an endothelial surface marker (Figure 1). The SLCO2A1 positive cells were exactly CD31 positive cells. The results confirmed our previous hypothesis in Year 2 that SLCO2A1 is an endothelial cell-specific SLCO transporter. SLCO5A1 was found to be exclusively expressed in the cancer cells and epithelial cells in this project. The contrasting cell type specificities of the two SLCO transporters suggest distinctive biological functions of the transporters; therefore have potential impact on prostate cancer biology. More important, the difference in cell type specificity coincided with the difference in the associations of the transporters with prostate cancer characteristics that was discovered in Aim 1.

The differential expression pattern of SLCO2A1 and SLCO5A1 in the prostate is an absolutely novel finding and has never been reported in the literature. The results may shed light on new mechanisms for the race-specific associations observed in Aim 1. Therefore, before moving to evaluating expression of SLCO5A1 and SLCO2A1 in the invaluable RPCI 92 EA/AA TMA, we further validated the expression of SLCO2A1 and SLCO5A1 using a TMA contains 36 matched benign and cancer tissue specimens of the prostate as part of the follow up study. This validation study also provides training of lab staff for visual scoring of IHC stained TMA cores. Our visual scoring method involves 3 individual scorers, each independently scores all the cores of the TMA sections, with 100 cells from each core and hundreds of
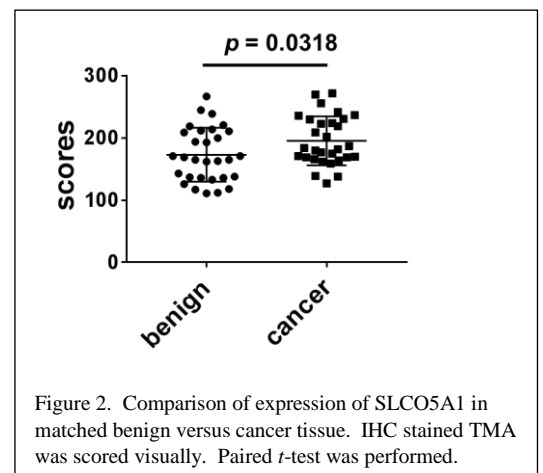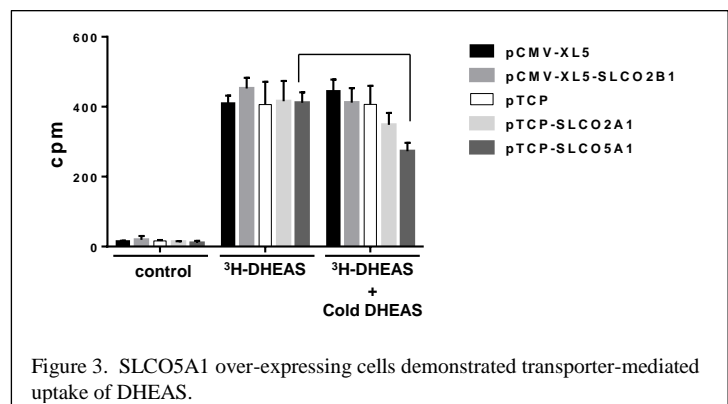

Figure 2. Comparison of expression of SLCO5A1 in matched benign versus cancer tissue. IHC stained TMA was scored visually. Paired t-test was performed.

cores per TMA section. This protocol is labor- and time-consuming but provides more reliable results compared to other methods. Therefore, training of scorers is critical to the quality of the data. Finding of this follow up study is summarized in Figure 2. When compared between paired benign and cancer tissue cores, the expression of SLCO5A1 was significantly higher in cancer than in adjacent benign tissue ($p < 0.05$). This finding was in agreement with the finding in Aim 1 that SNPs in SLCO5A1 were associated with high clinical stages. IHC staining for SLCO5A1 is ready to move forward. We have obtained the 92 AA/92 EA TMA sections and the staining has been finished in the beginning of October, 2017. Visual scoring and summary of data are expected to be competed in the first 3-5 months in the 1-year EWOF.

Due to the exclusive endothelial-specific expression of SLCO2A1, IHC staining instead of RNAScope staining may provide better signal for visual scoring. This is because endothelial cells tend to be much smaller than epithelial cells or cancer cells, which may lead to a grainier texture of RNAScope staining and impede accurate scoring. Another concern was on the age of the cores used for constructing the TMA. Over the past 2 years we have accumulated sufficient experience in RNAScope, and we noticed that TMAs with aged cores tend to detach and the staining signals tend to fade. Additional factors include the age of the sections of the TMA. Sections that are 6-month to 1-year old tend to have weakened signals. TMA sections are usually precut and stored for considerable long time. Due to these considerations, whenever an antibody is available the IHC may provide results of better quality from these particular sections. Unfortunately, SLCO2A1 antibody was repeatedly put on back order by the manufacturer during Year 3. The recent updated available date for this order was November, 2017. As a backup plan to ensure the completion of the SLCO2A1 study, a custom SLCO2A1 antibody was ordered from Pacific Immunology, an antibody service company. The antibody is raised against SLCO2A1 epitope and is purified using the same epitope. This order is on schedule and the final product will be available in the middle of October, 2017. Overall, it may take 4-7 months to complete IHC staining of SLCO2A1 on the 92AA/92EA TMA. Based on the results, we may need to request TMA sections from PCaP study for validating findings of SLCO5A1 and SLCO2A1 IHC staining. An additional 6-8 months is needed for staining and visual scoring. Since 3-4 months are needed for summarize the final data and for preparing and revising a manuscript for peer-reviewed publication, the total time needed for completion of all the IHC studies will be 12 months at most.

Aim 3. Characterize functions of candidate SLCO transporters in androgen uptake and evaluate the biological effects on AR signaling in human prostate cancer cell lines. Originally this aim was design to be guided by findings in both the genotyping results in Aim 1 and the expression levels on TMA in Aim 2. The original plan was based on the prediction that multiple SLCO transporters may be identified in the genotyping study. Since the actual findings in genotyping revealed that SLCO5A1 and 2A1 may be the most promising transporters, cell biology studies were

Figure 3. SLCO5A1 over-expressing cells demonstrated transporter-mediated uptake of DHEAS.

carried out for the 2 transporters without results from the TMA studies. SLCO5A1, 2A1 and 2B1 were transiently over-expressed in a human prostate cancer cell line LAPC-4. SLCO2B1 was included in the experiment because of its similar cell type specificity shared with SLCO2A1. The transfected cells were tested for the uptake of ^{3}H-dehydroepiandrosterone sulfate (DHEAS). Non-radioactive DHEAS, or so-called cold DHEAS, was added in 300-fold excessive amount for competition with ^{3}H-DHEAS. Transporter-specific uptake of DHEAS would be indicated by the reduction of the radioactive in the cell lysate in the presence of

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cold DHEAS (Figure 3). The reduction occurred in the SLCO5A1 over-expressing cells. This preliminary finding indicated that DHEAS may be a substrate for SLCO5A1, but not for SLCO2A1. This observation echoes the different expression pattern of SLCO2A1 and 5A1 in the prostate (Aim 2) and the race-specific associations of genetic variants in SLCO2A1 and 5A1 with prostate cancer characteristics (Aim1). Currently this finding is being verified in other cell lines. If the finding is confirmed, then SLCO5A1 may indeed play a very important role in intracellular T/DHT production in cancer cells, because our other data showed that DHEAS is a substrate for intratumoral T/DHT production (data not shown).

The experiments to test the role of SLCO5A1 or 2A1 in cell growth or AR signaling is ongoing. Stable clones that over-express the transporters were the optimal choice for these tasks. This is because transiently transfected cells are not suitable for long-term experiments such as growth, and inconsistent transfection efficiency may cause inconsistent results that is further worsened by double transfection involved in luciferase-based promoter-reporter assay for AR activity. The over-expressing plasmids for the SLCO transporters used puromycin resistance as selection marker for establishing stable clones. We established puromycin sensitivities of human prostate cancer cell lines used for the proposed work. We are in the process of establishing stable clones for the biological studies.

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

In year 3, one of the biggest gain for Dr. Wu is to witness the evolving progression of the analysis of the genotyping data, led by Dr. Tang and Dr. Zhu. As a laboratory-based basic science researcher by training, Dr. Wu would not have had the opportunity to learn from the two co-investigators on the sophisticated statistical models that were applied to the different data and different study questions. This was the first time for Dr. Wu to interact closely with a molecular epidemiologists and a biostatistician on large-scale data analysis. The experience is invaluable. A lesson that Dr. Wu learned from the interaction with Dr. Mohler and Dr. Azabdaftari was on the complicated issues revolving around the use of TMA, and how important the expertise of a pathologist is to the research using TMA. Dr. Azabdaftari would inspect each core to verify its pathological characteristic, and to exclude unreliable staining caused by misclassifications of the core, or by missing cancer cells in a cancer core. This process refreshed Dr. Wu's previously over simplified view that only relies on the clinical pathological reports for core classification.

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?

1) The genotyping and data analyses have been completed during the funding period. We are in the process of preparing a manuscript for a peer-reviewed publication, which may need 3- 6 months for submission and revision pending the review.

2) Complete IHC staining of SLCO5A1 and SLCO2A1 in the RPCI 92 AA/92 AA TMA, and to request TMA sections from the PCaP study for the IHC staining if necessary. The IHC of SLCO5A1 has been finished in the beginning of October, 2017. The visual scoring will follow and will be finished in 3-5 months. The IHC of SLCO2A1 was delayed due to recurring backorder of the antibody by the manufacturer. The last updated available date for the antibody was November, 2017. A new SLCO2A1 antibody is being raised by Pacific Immunology, an antibody service company. The antibody will be available in October, 2017. Overall, it may take 4-7 months to complete the SLCO2A1 IHC on the 92AA/92EA TMA. Based on the results, we may need request TMA sections from PCaP study for validating findings of SLCO5A1 and SLCO2A1 IHC staining. An

additional 6-8 months is needed for staining and visual scoring. Since 3-4 months are needed for summarize the final data and for preparing and revising a manuscript for peer-reviewed publication, the total time needed for completion of all the IHC studies will be 12 months at most.

3) Genotyping results indicated that SNPs that were associated with prostate cancer characteristics in EA and AA were intronic. This finding complicated the functional study. The intronic genetic variants may affect gene splicing, which results in different isoforms of the same gene. Evaluation of the functional significance of these genetic variants is fundamentally different from the evaluation of exonic genetic variants. Therefore, more time is needed to modify our biological systems accordingly. The function of the identified intronic genetic variants will be evaluated using the minigene approach. This approach will take 6-8 months due to the molecular biological steps for constructing the minigene splicing reporter constructs. Meanwhile, the biological functions of wild-type SLCO5A1 and SLCO2A1 will be finished and summarized in the next 2-3 months. Overall, 6-8 months are needed to complete this part of the study, and 3-4 months are needed for manuscript submission and revision.

4. IMPACT
What was the impact on the development of the principle discipline(s) of the project?

Nothing to report.

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.

Year 3 was the most challenging year through the whole funding period. A couple of unexpected events delayed the projects. The first was the back order of the SLCO2A1 antibody. This antibody was first ordered in November, 2016. However, the manufacturer had to back order multiple times. In order to avoid further, unpredictable delay, we identified Pacific Immunology among 5 antibody service companies to raise a custom antibody for SLCO2A1. The order is on schedule and antigen-specific affinity purified IgG will be delivered to us in October, 2017. The second event that caused the delay was the relocation of all research laboratories into a new building. The routine operations of the labs were disturbed for nearly 3 months due to the relocation. The IHC core that was led by Dr. Elena Pop was not resumed until recently. Also affected was the Aim 3 cell biology studies, especially the uptake experiments that require use of radioactive androgens. To minimize the impact, we finished the matched benign – cancer TMA using SLCO5A1 antibody and used the stained TMA to

train scorers who needed the least function of the lab, and managed to obtain data that showed SLCO5A1 was expressed at higher level in cancer versus benign tissue.

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.

Nothing to report.

6. PRODUCTS
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Yue Wu, Ph.D. (2 cal months) – PD/PI

Li Tang, Ph.D. (1 cal month) – Co PD/PI

James Mohler, M.D. (<1 cal month) – Co-I

Gissou Azabdaftari, M.D. (1 cal month) – Co-I

Qianqian Zhu, Ph.D. (1 cal month) – Co-I

Elena Pop (1 cal month) – Research Associate

Todd Parsons (2 cal month) – Technician

Rachel Pratt (1 cal months) – Technician

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

Yes. Updated active other supports of Dr. Yue Wu (PI), Dr. James Mohler (co-I), Dr. Gissou Azabdaftari (co-I), Dr. Qianqian Zhu (co-I) and Dr. Elena Pop (co-I) are presented as follows. Nothing to report for Dr. Li Tang (co-PI).

Changes in active support

Wu, Y.
Active to Completed
Title: Deprive prostate cancer of DHEAS to prevent castration-recurrent prostate cancer (Wu)
Time Commitments: 2.40 calendar months
Supporting Agency: NIH/NCI (1R21CA191895-01)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Viviana Knowles, Grants Management Specialist, 9609 Medical Center Drive, West Tower, Bethesda, MD 20892, phone: 240-765-5157, viviana.knowles@nih.gov
Performance Period: 09/17/2014-08/31/2017 (nce)
Level of Funding: $419,884

Brief description of project's goals: This research seeks to address the racial differences in prostate cancer aggressiveness from a biological perspective.
List of specific aims:
Aim 1. Characterize the expression of STS and potential STS regulators in CRPC
Aim 2. Evaluate the value of targeting DHEAS usage by prostate cancer cells to prevent post-castration tumor growth
Aim 3. Identify DHEAS uptake mechanisms
Overlap: None

Mohler, J.L.
Pending to Active
Title: Genetic and Epigenetic Prostate Cancer Related alterations in early onset disease in African American Men (Woloszynska-Read)
Time Commitments: 1.20 calendar months
Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Department of Defense, USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014/ LYMOR BARNHARD
Performance Period: 04/01/2017-03/31/2020
Level of funding: $1,242,951
Brief description of project's goals: Proposed research aims to identify molecular alterations that distinguish aggressive forms of early onset prostate cancer commonly found in Afrinca American men will contribute to the development of African American tumor (epi)genetic signature(s) and ultimately will lead to personalized medicine strategies for this group of patients.
List of specific aims:
1. Determine the relative frequency of genetic lesions found in PCa in AAs and EAs.
2. Determine novel, clinically relevant methylomic and transcriptomic differences in PCa from AAs and EAsObtain and link vital status data and cause of death in PCaP research subjects
Overlap: None

Title: Racial differences in financial impact of prostate cancer treatment and outcome (Mohler)-Recommended for funding
Time Commitments: 1.44 calendar months Y1, 1.8 calendar months Y2, 2.40 calendar months Y3
Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Not assigned
Performance Period: 07/01/2017-06/31/2020
Level of funding: $445,328
Brief description of project's goals: Recurrence of advanced CaP during androgen deprivation therapy leads to a variety of new FDA-approved treatments, which may include immunotherapy, androgen metabolism inhibitors, small molecule anti-androgens, radio-pharmaceuticals, and docetaxel, cabazitaxel or cisplatin, all of which can extend survival but cause side effects and are expensive. Complexities of insurance coverage and Medicare reimbursement, a trend toward increasing co-pays for covered medications and differences in availability of financial assistance from pharmaceutical companies for new agents makes challenging the anticipation of the amount of financial burden posed by advanced CaP. If cured of localized CaP, costs may result from treatment of side effects, such as incontinence and impotence. CaP has been reported to produce the highest level of financial distress among 7 common cancers studied. Patients and their family members have suffered loss of their home, had to quit or decrease job hours or intensity, or forego expensive treatments shown to prolong life. AAs compared to Caucasian Americans (CAs) have been reported to benefit from higher religiosity and "caregiverness" but suffer from lower socioeconomic reserve and medical sophistication. The central hypothesis is that the financial impact of CaP treatment and oncologic outcome differs between AAs and CAs newly diagnosed with CaP.
List of specific aims:

1. Locate and contact PCaP research subjects to update CaP status, CaP treatments received and comorbidities, repeat the QoL assessments performed at baseline and follow-up, and administer new surveys on financial burdens and stress and caregiver QoL and support
2. Locate and contact PCaP research subjects' treating physicians to update treatments received and oncologic outcome data
3. Obtain and link vital status data and cause of death in PCaP research subjects
4. Examine the role financial burden and stress have on CaP survival and QoL and whether this relationship was modified by race.

Overlap: None

Active to Completed
Title: Deprive prostate cancer of DHEAS to prevent castration-recurrent prostate cancer (Wu – PI)
Time Commitments: 0.12 calendar months
Supporting Agency: NIH/NCI 1R21CA191895-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Viviana Knowles, 9609 Medical Center Drive, West Tower, Bethesda, MD 20892, phone: 240-276-5157, viviana.knowles@nih.gov
Performance Period: 09/17/2014-08/31/2017 (NCE)
Level of Funding: $419,884
Brief description of project's goals: This research seeks to address the racial differences in prostate cancer aggressiveness from a biological perspective.
List of specific aims:
1. Characterize the expression of STS and potential STS regulators in CRPC
2. Evaluate the value of targeting DHEAS usage by prostate cancer cells to prevent post-castration tumor growth
3. Identify DHEAS uptake mechanisms

Overlap: None

Title: Prostate Cancer: Transition to Androgen Independence, Project 1: Interference with the Androgen Receptor and Its Ligands in Recurrent Prostate Cancer (French - PI)
Time Commitments: 0.60 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295
102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2017 (NCE)
Level of Funding: $2,292,618
Brief description of project's goals: Renewal of a project that tests the hypothesis that recurrence of prostate cancer during androgen deprivation therapy can be prevented or delayed by preventing the accumulation of tissue androgens and/or inhibiting the androgen receptor.
List of specific aims:
1. Prevent the changes in androgen metabolism that provide AR ligand(s) in the immediate post-castration period
2. Degrade AR ligand(s) formed in the immediate post-castration period
3. Diminish or eliminate AR in the immediate post-castration period

Overlap: None

Title: Diet changes among prostate cancer patients under expectant management (Marshall - PI)
Time Commitments: 0.60 calendar months
Supporting Agency: National Cancer Institute
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Program Official:
Howard L. Parnes, Email: hp24c@nih.gov Phone: 301-594-0920 Fax: 301-435-1564

Performance Period: 09/28/2009-01/31/2017 (NCE)
Level of Funding: $55,818
Brief description of project's goals: The focus of this study is to assess whether a diet emphasizing plant consumption decreases the probability that low grade, low-volume prostate cancer (LGLV)in expectant management(EM)patients progresses to a more aggressive form of cancer that merits active treatment. The intervention will be conducted through one of the leading cooperative oncology research groups: Cancer and Leukemia Group B (CALGB).
List of specific aims:
1. Assess the effect of a telephone-based dietary intervention on PSA, PSA doubling time, Gleason score and tumor extension in LGLV prostate cancer patients treated with EM.
2. Assess the effect of a telephone-based dietary intervention on treatment seeking, anxiety and coronary heart disease in prostate cancer patients treated with EM.
Overlap: None

Azabdaftari, G.
Pending to Active
Title: Genetic and Epigenetic Prostate Cancer-Related Alterations early-onset Disease African American men
Time Commitment: 1.20 Calendar Months (PI, Woloszynska-Read)
Supporting Agency: DOD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Performance Period: 04/01/2017-03/31/2020
Funding: $865,000
Brief description of project's goals: Our working hypothesis is that molecular alterations not currently determined, such as ETS fusions, tumor suppressor promoter hypermethylation, and gene body methyl-ation, are distinct between African Americans and European Americans, contributing to early onset of PCa and more aggressive disease in AAs. We will use whole genome bisulfite sequencing (WGBS), deep-coverage paired-end RNA sequencing, Illumina 450K DNA methylation array, and pyrosequencing.
Specific Aims:
1. Determine the relative frequency of common genetic lesions found in prostate cancer in tumors from African Americans and European Americans. Our working hypothesis is that AAs display a PCa mo-lecular profile with TMPRSS2-ERG fusion frequency, PTEN deletion, ERG, SPINK1 and AR expression distinct from their EA counterparts. Moreover, this Aim will integrate analysis of AR expression with the other types of lesions just mentioned for the first time. We will use a combination of tissue microarrays (222 AAs; 249 EAs), fluorescence in situ hybridization, and immunohistochemistry.
2. Determine new, potentially relevant methylomic and transcriptomic differences in tumors from African Americans and European Americans. Our working hypothesis is that molecular alterations not currently determined, such as ETS fusions, tumor suppressor promoter hypermethylation, and gene body methyl-ation, are distinct between African Americans and European Americans, contributing to early onset of PCa and more aggressive disease in AAs. We will use whole genome bisulfite sequencing (WGBS), deep-coverage paired-end RNA sequencing, Illumina 450K DNA methylation array, and pyrosequencing.
Overlap: None

Active to Completed
Title: Therapeutic Efficacy of Riluzole in Prostate Cancer Cancer (1R21CA181152-01A1)
Time Commitment: 0.30 Calendar Months (PI, Koochekpour)
Supporting Agency: NCI
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Michael C. Alley
Performance Period: 07/01/2014 – 06/30/2017
Funding: $221,589
Brief description of project's goals: This is a translational prostate cancer research aimed at determining the effect of glutamate receptor antagonist on tumor growth and metastatic ability, fatty acid synthase (FAS)

expression and apoptotic markers in prostate cancer. This study will investigate the underlying mechanisms by which glutamate receptor antagonist down regulates FAS expression in prostate cancer cell lines.

Role: Co-Investigator (Pathologist)

Overlap: None

Title: Deprive Prostate Cancer of DHEAS to Prevent Castration-Recurrent Prostate Cancer (1R21CA191895-01)

Time Commitment: 0.60 Calendar Months (PI, Wu)

Supporting Agency: NIH

Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Neeraja Sathyamoorthy

Performance Period: 09/17/2014 – 08/31/2017

Funding: $229,028

Brief description of project's goals: To investigate the role of dehydroepiandrosterone sulfate (DHEAS) in the progression of prostate cancer to castration-recurrent prostate cancer. The goals are to identify the molecular mechanisms underlying the uptake, metabolism, ad biological functions of DHEAS, and to identify potential targets to deprive prostate cancer cells of DHEAS to prevent the progression tc astration-recurrent prostate cancer.

Specific Aims:

1. Characterize the expression of STS and potential STS regulators in CRPC.
2. Evaluate the value of targeting DHEAS usage by prostate cancer cells to prevent post-castration tumor growth
.
3. Identify DHEAS uptake mechanisms.

Role: Co-Investigator (Pathologist)

Overlap: None

Title: Metabotropic Glutamate Receptor 1 in African American Prostate Cancer (1R21CA183892-01)

Time Commitment: 0.30 Calendar Months (PI, Koochekpour)

Supporting Agency: NCI

Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Elizabeth Woodhouse

Performance Period: 04/01/2014 – 03/31/2017

Funding: $184,658

Brief description of project's goals: The objective of this project is to investigate the expression of metabotropic glutamate receptor and its association with clinical progression in a large cohort of African Americans with PCa.

Specific Aims:

1. To determine the association between tissue expression of GRM1 and clinicohistopathological predictors or prognosticators of PCa progression or aggressiveness in AAs.
2. To determine the association between GRM1 expression levels and invasive and metastatic phenotypes in AA-PCa cells. Data generated from this exploratory study will define biological and/or clinicohistopathological significance or relevance of GRM1 expression in AA-PCa and may prove useful in discriminating clinically or biologically aggressive tumors from indolent (non-aggressive) tumors and minimizing PCa disparity in AAs.

Role: Co-Investigator (Pathologist)

Overlap: None

Zhu, Q.

Pending to Active

Title: The Role of TAZ in Breast Cancer Initiation and Progression (1 R01 CA207504-01A1)

Time Commitments: 0.60 calendar (PI-Zhang)

Supporting Agency: NIH

Grants Officer: Pending

Performance Period: 07/01/17-06/30/22

Level of Funding: $2,188,322

Brief Description of Project's Goals: Our long-term goal is to understand the mechanisms of TNBC relapse and thus help to improve the survival of breast cancer patients. Our overall objective here, which is the next step

in pursuit of that goal, is to determine how TAZ activation induces TNBC tumor progression and metastasis. Our central hypothesis is that both TAZ-dependent cell cycle activation and expansion of transformed mammary stem cell (Ma-SC) populations are required for TAZ-initiated breast tumorigenesis. Our hypothesis has been formulated on the basis

List of Specific Aims:
1. Identify the critical downstream targets that are required for TAZ-initiated oncogene addiction.
2. Determine how TAZ induces the formation of heterogeneous mammary tumors using a unique TAZ transgenic mouse model.
3. Determine the role of TAZ in breast tumor heterogeneity and its impact on tumor metastasis.

Overlap: NONE

Active to Completed
Title: Genomic markers predicting tumor response to cytotoxic chemotherapy (1R01 CA202354-01)
Time Commitments: 0.00 calendar (PI-Demant)
Supporting Agency: NIH
Grants Officer: Sudhir B. Kondapaka; sudhir.kondapaka@nih.gov; (240) 276-5365
Performance Period: 12/1/15-11/30/17
Level of Funding: $50,000
Brief Description of Project's Goals: We propose to develop a novel way to determine in advance whether individual cancer patients will benefit from a therapy with a certain anti-cancer drug, or whether they should receive another drug, because their tumor is not likely to be suppressed by the drug considered as the first. The specific advantage of the method we propose is that it is based not only on the current knowledge of pharmacology of anti-cancer drugs, but can discover also reliable predictive factors that are based on novel mechanisms.

List of Specific Aims:
1. Determination of linkage of Tctr genes polymorphic between CcS-2 and CcS-9 will be performed by standard linkage methods in F2 hybrids using a whole polymorphic genome coverage.
2. The linkages detected in the previous experiment will be confirmed in subsequent backcrosses that will serve as starting points for production of congenic lines, each carrying a single Tctr gene, so the functions of each such gene could be investigated separately. However, these congenic lines cannot be completed within the time frame of this project.

Overlap: NONE

Title: Antiretroviral Pharmacogenetics, Pharmacokinetics and Toxicity in Neuroaids (5K08MH098794-04)
Time Commitments: 0.00 calendar (PI-Ma)
Supporting Agency: NIH
Grants Officer: Colosi, Deborah Ann; deborah.colosi@nih.gov; Office: (301) 605-2275
Performance Period: 7/1/12-6/30/17
Level of Funding: $111,052
Brief Description of Project's Goals: The research plan focuses on the development of a model system to improve risk and intervention assessments by integrating genetic data, pharmacokinetics and toxicity to establish an individualized risk profile of HIV-associated neurocognitive disorders, one of the prevalent co-morbidities in treated individuals.

List of Specific Aims:
1. To determine the association between antiretroviral pharmacokinetics and neurocognitive function among treated patients from CHARTER and Chinese studies.
2. To identify genes and genetic polymorphisms that are associated with antiretroviral exposure, particularly genes that are linked to drug metabolism and drug distribution into the central nervous system.
3. To identify neurotoxicity and inflammation-associated genes that are linked to neurocognitive abnormalities using gene expression profiling and bioinformatics techniques.

4. To develop a disease progression model that integrates pharmacokinetics and the genetic data generated from aims 1 to 3 to predict HAND development.

Overlap: NONE

Pop, E.
Pending to Active
Title: Genetic and Epigenetic Prostate Cancer Related alterations in early onset disease in African American Men (Woloszynska-Read)
Time Commitments: 1.20 calendar months
Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Department of Defense, USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014/ LYMOR BARNHARD
Performance Period: 04/01/2017-03/31/2020
Level of funding: $1,242,951
Brief description of project's goals: Proposed research aims to identify molecular alterations that distinguish aggressive forms of early onset prostate cancer commonly found in Afrinca American men will contribute to the development of African American tumor (epi)genetic signature(s) and ultimately will lead to personalized medicine strategies for this group of patients.
List of specific aims:
3. Determine the relative frequency of genetic lesions found in PCa in AAs and EAs.
4. Determine novel, clinically relevant methylomic and transcriptomic differences in PCa from AAs and EAsObtain and link vital status data and cause of death in PCaP research subjects
Overlap: None

Active to Completed
Title: Deprive prostate cancer of DHEAS to prevent castration-recurrent prostate cancer (Wu)
Time Commitments: 1.80 calendar months
Supporting Agency: NIH/NCI 1R21CA191895-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Viviana Knowles, 9609 Medical Center Drive, West Tower, Bethesda, MD 20892, phone: 240-276-5157, viviana.knowles@nih.gov
Performance Period: 09/17/2014-08/31/2017 (NCE)
Level of Funding: $466,950
Brief description of project's goals: This research seeks to address the racial differences in prostate cancer aggressiveness from a biological perspective.
List of specific aims:
Aim 1. Characterize the expression of STS and potential STS regulators in CRPC
Aim 2. Evaluate the value of targeting DHEAS usage by prostate cancer cells to prevent post-castration tumor growth
Aim 3. Identify DHEAS uptake mechanisms
Overlap: None

Title: Prostate Cancer: Transition to Androgen Independence, Core B: ImmunoAnalysis and Tumor Management (French – PI)
Time Commitments: 9.0 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295, 102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2017 (NCE)
Level of Funding: **$903,203**

Brief description of project's goals: Renewal of a core that serves two primary functions to the three projects: Core B is involved in all aspects of clinical specimen and prostate cancer xenograft management and Core B processes and stores the invaluable prostate biopsy specimens obtained from men with advanced prostate cancer prior to and at regular intervals after beginning androgen deprivation therapy.

List of specific aims:
Aim 1. Core B will provide high quality, reliable and cost-effective technical services to participants of the Program Project for immunohistochemistry and quantitative image analysis.
Aim 2. Core B will manage the research specimens critical to the conduct of the research proposed by the Program Project.
Aim 3. Core B will provide expertise in biostatistics and genitourinary pathology.

Overlap: None

What other organizations were involved as partners?

Nothing to report.