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TITLE: Molecular Markers of Estrogen Metabolism and Progression from High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) to Prostate Cancer

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The purpose of this case-control study is to investigate the association between genetic and encocrine markers of estrogen metabolism and prostate cancer progression. Androgens (e.g., testerone) may be critical in prostate cancercinogenesis, but there is accumulating evidence that estrogens facilitate progress during the later stages of prostate cancer formation (1-4). To explore the role of estrogens in human prostate carcinogenesis, we proposed to investigate the association between genetic and endocrine markers of estrogen metabolism and the detection of high-grade prostatic intraepithelial neoplasia (HGPIN) and stage I/II/III prostate cancer. While the first project year included protocol development and IRB approval, the second year of this study focused on subject recruitment and data collection. Specific accomplishments include recruitment of 146 subjects to the protocol (85% of eligible), with complete data collected form 129 subjects. We have expanded the recruitment base to the Veterans Hospitals, refined data collection materials, and are pilot testing our laboratory procedures. We anticipate successful project completion, and further details provided below are in parallel with the statement of work.

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MOLECULAR MARKERS OF ESTROGEN METABOLISM AND PROGRESSION FROM HIGH-GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA (HGPIN) TO PROSTATE CANCER

Second Annual Report

This study has completed the second full year of funded activity. Data collection protocols have been finalized and protocols approved by appropriate IRBs. The majority of work revolved around subject recruitment, data collection, and biospecimen collection necessary to achieve specified aims. Specific accomplishments are described in the following narrative, in parallel with the original Statement of Work.

INTRODUCTION

This work is based on a growing body of laboratory evidence that estrogens contribute to the advancement and detection of prostate cancer. Prostate tumor cells express estrogen receptors, and estrogen exposure may sustain tumor growth as androgen levels decrease with advancing age (1). It has further been shown that not all estrogens behave alike. Relative to 2-hydroxyestrone (2HE), 16HE appears bind with higher affinity to the estrogen receptor (5), and the relative balance of estrogen metabolites is determined by several enzyme activities. The CYP3A4 and CYP1B1 P-450s are responsible for converting estradiol to the 16HE or 4 HE metabolites, respectively, at the expense of 2HE production. Both CYP1B1 and CYP3A4 are expressed in prostate tissue, a polymorphism of the *CYP3A4* gene in the 5' transcriptional regulatory element (*CYP3A4-V*) has been significantly associated with later age and advanced stage at CaP detection compared to men with the wild-type gene (6). Similarly, prostate cancer cases may be 3-fold more likely than controls to be homozygous for the *CYP1B1* ^{Val} allele (7). The goal of this New Investigator Award is to conduct a pilot case-control study investigating the association between molecular markers of estrogen metabolism and diagnosis of prostate cancer (stage II/III) and high-grade prostatic intraepithelial neoplasia (HGPIN), the purported precursor pathologic state to prostate cancer. We will recruit 45 men with HGPIN, 45 prostate cancer cases, and 45 healthy controls free of prostate cancer at biopsy. Genetic polymorphisms in CYP3A4 and CYP1B1 associated with estrogen metabolism will be determined from blood collected from each participant. Data regarding family history of prostate cancer and other risk factors is collected by questionnaire, and habitual dietary intake is measured by in-person interview. We hypothesize that CaP cases will have lower urinary 2HE/16HE and 2HE levels, and higher urinary 16HE, 4HE, E2 and blood E2 levels compared to HGPIN cases or healthy controls.

With completion of the second year of this 3-year award, we have met and built upon the 1-Year objectives specified in our statement of work, including the obtainment of IRB approval, development of questionnaires and other data collection tools. We have expanded our recruitment base to include the Veteran's Hospitals in Nashville and Murfreesboro, TN. Recruitment of study participants has required the most work, and recruitment will continue into the third year. Also in

the third year, data will be processed for analysis, blood and urine specimens analyzed for described markers, and our analyses will determine the association between estrogen-related markers and prostate cancer.

BODY

Work accomplished or initiated during the Year 1 and Year 2 of this NIA encompasses Statement of Work tasks outlined in Stage 1 (Run-In: Months 1-8)) and Stage 2 (Recruitment and Data Collection: Months 9-26)), Stage 3 (Months 12-30), and Stage 4 (24-30 months). Work outlined as Stage 5 has not been initiated.

Stage 1: Run-In Phase: Months 1-8:

1. Develop all questionnaires, pathology record abstraction forms, and data collection protocols

   Year 1: All forms were developed specifically for this project. Our risk factor questionnaire measures include: demographics; age; sex; race; marital status; education; number of children; medical history and treatment; family health history of prostate cancer; history of other cancers, exercise frequency; and smoking status. (Appendix). Our dietary assessment instrument is the Diet History Questionnaire, recently developed by the NIH and shown to have favorable reliability and validity to other established dietary assessment instruments. We had a computer-assisted program developed to guide the implementation of our dietary assessment instrument. Our Pathology Record Abstraction form records the clinic sites, dates and clinical results of all PSA, other lab assays, and digital rectal exam results, ultrasound results, biopsy history and results, pathological diagnosis (HGPIN, cancer), and zone and distribution of the disease. Similarly, we record any cancer diagnosis, as well as stage and Gleason scale of cancer. We have developed Body Measurement protocols (Anthropometrics), including the methods of measuring and recording measurement of participant weight, height, sitting height, waist, and hips. Our Biospecimen Collection form records blood collection status for purposes of DNA extraction or serology, and urine specimens for estrogen metabolite measurement. All samples are aliquated and stored frozen as necessary to meet our research objectives. Medication use is recorded on the Medication Use form. Subjects are instructed to bring all current medications and supplements to the data collection meeting, and name of the drug, dosage, and use patterns are recorded. The Interview Checklist ensures that all assigned tasks required during the interview are completed, including a review of eligibility, completion of all data collection protocols, and proper consent of participant.

2. Hire a Research Nurse

   Year 1: For this project, Saundra Motley, RN, was hired to serve as Project Manager. Ms. Motley is responsible for the day-to-day administration. She also has been trained to collect body size measurements using standard and systematic protocols, as well as in urine and blood collection, sample preparation, and storage protocols.

Year 2: Ms. Motley continued to serve as Project Manager. She is responsible for recruitment, data collection, and day-to-day administration of the project.

3. Pre-test questionnaires and forms

   Year 1: All instrument materials have been thoroughly reviewed, and final versions of all assessment instruments have been produced.

4. Refine questionnaire protocols, as necessary

   Year 1: The Baseline Questionnaire was edited to provide a better organization to the questions. We have improved our assessment of dietary intake by developing portion size guides (Appendix), and the addition of several foods common to the Tennessee population (asparagus, okra, squash). We are conducting a sub-study to determine if completion of the dietary questionnaire at home and with a wife/partner provides substantially different food intake scores compared to our in-person interview protocol. The laptop computer program we had developed for this project initially had several 'glitches', but is now working without errors or failures.

5. Finalize IRB application and consent form, if necessary

   Year 1: IRB approved consent forms have been obtained from Vanderbilt University, the VA (Nashville), and the US AMRMC (Appendix).

   Year 2: We have started to expand the recruitment base to the VA in Murfreesboro TN. Also, we are in negotiations with a large private urology practice to start recruitment at this site. If this appears feasible, a DOD representative will be notified.

6. Develop the study data management systems, including the combination of Teleform, Microsoft Access, Epi-Info, and SAS

   Year 1: Our data management system continues to develop, as we recognize and incorporate new technologists into our study. We are able to rapidly identify potentially eligible men receiving care at one of the urology cancer centers through newly implemented scheduling and patient monitoring software in the clinic. This will greatly increase our efficiency to recruit urology patients in the future. New data collection protocols have been developed to fully utilize all resources. Dietary assessment data are entered directly into an ACCESS database using a computer-based dietary assessment interview program created specifically for this project. The ACCESS database may be read by SAS, and merged with other databases for the purposes of analysis. Additional data-entry programs for questionnaires and forms will follow this model, using ACCESS with subsequent transfer into SAS.

   Year 2: Data entry programs for all data collection forms have been developed. Data are entered on a periodic basis throughout the year. About 50% of collected data have been key-punched at this time.

7. Become trained in all clinic-site office and administrative procedures

Year 1: The PI and/or Project Manager have received tours of the urology clinic at Vanderbilt and the VA (Nashville), and understand the operational and administrative procedures of this clinic.

Year 2: The PI and Project Manager have received tours of the urology clinic at the VA (Murfreesboro), and recruitment at this site has started.

8. Hire and Train Project Director in all clinic based procedures

Year 1: See #2 above. The position of Project Director and Research Nurse were efficiently combined under Ms. Motley's position in the study.

Year 2: See #2 above. Ms. Motley continues as Project Coordinator. With expansion of recruitment to the VA at Murfressboro, TN, she is training a VA research nurse in our data collection protocols, in addition to other study responsibilities. Full recruitment from this VA will begin once training is concluded.

9. Finalize all biological sample collection and storage procedures to be used in the study

Year 1: All biological sample collection and storage procedures for urine and blood cells and serum are finalized. After obtaining consent, we obtain a urine and blood sample, body size measurements, and participants complete the diet questionnaire. Urine is aliquoted to 9 cryovials, and stored at –80 C. One tube of blood, 5ml (EDTA) is collected for DNA extraction.Two 5ml tubes of blood (No anticoagulants) are collected, centrifuged, and the supernate aliquoted for steroid hormone measurement.

Year 2: Biospecimen collection and storage protocols are being implemented according to protocols.

10. Establish reliability of all laboratory procedures to be used

Year 1: The genotyping assays for CYP3A4 and CYP1B1 are routine genotyping assays in our group with established reliability. The estrogen metabolite assays have published reliability and validity. Laboratory reliability will be determined at the time the assays are scheduled to run, during Year 2 or Year 3 of the project.

Year 2: A subset of urine samples have been sent to Dr. Fritz Parl for trial analysis of urinary estrogen metabolite levels. Results from this lab trial were not available at the time of this report.

11. Establish screening procedures for men with HGPIN, prostate cancer patients, or controls.

Year 1: Candidates are identified by collaborators in the urology clinics as potentially eligible for our research study. As part of standard recruitment procedures, we mail an introductory letter and brochure to candidates (Appendix). This is followed by a telephone calls (Appendix for script) intended to answer any questions and evaluate interest in the study. If interested and determined potentially eligible, a meeting is scheduled at the study center. The Project Manager confirms eligibility and obtains informed consent (Appendix for consent form) prior to data collection.

Year 2: Recruitment and eligibility screening procedures were implemented.

12. Order supplies necessary for biological sample collection

    Year 1: All supplies necessary for biological sample collection have been ordered and received.

    Year 2: Additional supplies ordered as needed.

13. Create complete manual of operations

    Year 1: We have created a manual of operations to organize and record every procedures and protocol in this study. All items in the Appendix are included in the Manual, as well as a copy of the original grant proposal and correspondence with DOD colleagues.

    Year 2: The Manual of Operations continues to be updated as protocols are revised or as administrative documents are received.

Stage 2a: Recruitment of 45 men with high grade intraepithelial neoplasia, 45 men with Stage II/III prostate cancer, and 45 men without CaP or HGPIN at biopsy: Months 8-26

1. Identify 45 men with HGPIN eligible for the study from the urology clinics.
2. Identify 45 men with prostate cancer.

Year 1 and **Year 2**: Study recruitment started January 22, 2003. At the end of Year 1, we had recruited, consented, and collected complete data from 15 participants: HGPIN (n=3), Prostate Cancer (n=7), Controls (n=5). At the end of Year 2, we have recruited and obtained complete data from 129 subjects: HGPIN (n=12), Prostate Cancer (n=55), Controls (n=62). An additional 13 subjects are at various stages of recruitment and data collection, suggesting our likely recruitment for Year 2 will be near 142 subjects. At this time, 82% of patients approached for recruitment agree to participate, a reasonably high success rate in this day. We plan to continue recruitment into Year 3. We note that recruitment was initially delayed by approximately 4 months due to IRB requirements, thus a further extension of recruitment efforts to Year 3 should be expected. Furthermore, we note the difficulty in identifying patients with HGPIN. We knew it was the particular challenge for this study to identify these patients, and this challenge has led to extend recruitment to the VA at Murfreesboro, TN, and an attempt to extend recruitment to a large private urology medical practice in Nashville. Clinicians at both the Murfreesboro VA and the private medical practice inform us that HGPIN is more common among the patients at these new sites, and we expect to meet recruitment goal with once these additional recruitment sites come 'on-line'.

3. Abstract medical records for health history and pathology data, as described in Methods section of this proposal.

   Year 1 and **Year 2**: We have accessed medical records and abstracted relevant prostate cancer pathology information using our pathology data form.

4. Gain informed consent

   Year 1 and **Year 2**: All participants are consented in an IRB approved manner.

5. Among those who say they are willing to participate, determine eligibility using the criteria described in the Methods Section of this proposal

   Year 1 and **Year 2**: Eligibility is determined via telephone interview, and confirmed during the in-person interview.

6. Enroll consecutive eligible men with confirmed HGPIN or prostate cancer.

   Year 1 and **Year 2**: Approximately 82% of eligible HGPIN and prostate cancer patients approached for enrollment have enrolled in our study.

7. Ensure that the spot urine samples and blood samples are collected, processed, and stored in a –80° C freezer at the Vanderbilt University

   Year 1 and **Year 2**: All urine and blood samples are collected and processed using described protocols, then stored in a –80 C freezer at Vanderbilt University.

8. Collect data on lifestyle, demographics, and health (family and personal history), as outlined in proposal

   Year 1 and **Year 2**: Each participant completes a Background Questionnaire, collecting lifestyle, demographic, and medical data. This questionnaire is mailed to participants prior to the clinical visit, and then reviewed by the Project Manager at that time for completeness. Any blank questions or illogical responses are clarified at that time.

9. Pathology record abstractions will be performed

   Year 1 and **Year 2**: The Project Manager abstracts relevant medical and pathology information from the pathology report, and records this information on the Pathology Report Form. This data are periodically key-punched into an ACCESS database. We note that the study pathologist (Dr. Shappell) has taken a temporary leave-of-absence from work due to personal reasons. This has delayed confirmation of pathology data until he returns, and we expect this review completed in Year 3 of the project.

10. Collect anthropometrics

    Year 1 and **Year 2**: We measure weight, height, waist, hips, and sitting height for each participant using standardized protocols as described. All body measurements are recorded on our Body Measurement Form, and are periodically key-punched into an ACCESS data base.

<u>Stage 3: Data Entry, Verification and Interim Analysis, Months 12-30</u>

1. Assure that all data are immediately read into analytic databases

   Year 2: Data from the diet and physical activity interviews are immediately available for analyses. Data reported by questionnaire, or recorded on the appropriate forms, have been periodically key-punched into ACCESS databases, as work loads permit.

2. Flag all outlier and illogical responses

Year 2: All questionnaires are reviewed prior to data entry to identify possible illogical responses. At the time of this report, study recruitment and data collection is in progess. Once recruitment and data collection are closed, data verification and quality control procedures will be implemented.

3. Verify all outlier and illogical responses, re-contacting participants, if necessary.
4. Conduct simple descriptive analyses (e.g., cross-tabulations and univariate statistics)

Stage 4: Laboratory Analyses, Months 24 – 30

1. Transfer urine samples to the lab of Dr. Parl for estradiol and estrogen metabolite assays

Year 2: A subset of urine specimens have been transferred to Dr. Parl's lab for preliminary testing of endocrine assays. This will help us evaluate assay quality and variability when urine specimens, such that this analysis may be more rapidly completed for all subjects in Year 3 of the project.

2. Transfer blood sample to lab of Dr. Parl for estradiol assay
3. Transfer blood samples to lab of Dr. Cai for CYP1B1 and CYP3A4 genotyping assays
4. Confirm quality control procedures, and repeat assays if necessary

Stage 5: Final Data Analysis, Months 30-36:

- Perform exploratory analyses to test for adherence to model assumptions
- Perform any necessary data transformations to meet statistical assumptions
- Test study hypothesis
- Conduct post-hoc analyses of study data
- Prepare manuscripts
- Archive datasets for future analyses and future patient follow-up
- Plan for future studies

There has been no work accomplished within stage 5 of the Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS in Year 2

- Finalize and field test all questionnaires and data collection forms
- IRB approvals maintained
- Manual of Operations maintained
- Participant recruitment, including consent, recruitment, eligibility, data collection, biospecimen collection, and storage protocols.
- Extending recruitment to an additional VA, and in process with a private urology practice in Nashville.
- Abstraction of pathology data from medical records
- On-going data-entry of all data.
- Piloting urinalysis protocol for endocrine study outcomes

REPORTABLE OUTCOMES

This research has been used to demonstrate recruitment feasibility of prostate cancer patients for three grant applications. The first was submitted as a pilot project in a Prostate Cancer SPORE application submitted to the NIH. The objective was a case-control study extending this recruitment protocol to investigate additional biomarkers of prostate cancer risk (SPORE was not funded). In the second application, Dr. Fowke submitted a pilot intervention to the American Institute of Cancer Research to investigate the effects of diet on PSA levels among prostate cancer patients (unfunded after first submission, but scored well with positive comments and will be resubmitted). In the third project, Dr. Fowke will submit an IDEA award to the DOD to investigate the effects of indole-3-carbinol or Crucifer vegetable intake among men with PSA recurrence following prostatectomy.

CONCLUSIONS

In Year 2, recruitment and data collection protocols were fully implemented. We have complete data collection from 129 subjects, with data collection from 17 more in progress. We recruit 85% of eligible subjects to this study, a respectably high recruitment rate. We note that recruitment of HGPIN subjects has not occurred as rapidly as planned. This is due to initial delays with study start up due to issues around Human Subjects agreements, and a lower than expected patient flow through our early recruitment centers. To compensate, we have added recruitment from the VA at Murfressboro, TN, and full-scale recruitment from this center will begin as soon as a collaborating staff member in this center is adequately trained. Also, we are negotiating with another clinic for recruitment, and we will inform DOD if this negotiation is successful. To compensate for time initially lost, we are considering a request for a no-cost extension to meet study goals. Data entry is in progress, and quality control protocols for lab assays have begun. This NIA has provided the opportunity for Dr. Fowke to submit several prostate cancer research grants.

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