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TITLE: Imaging Prostatic Lipids to Distinguish Aggressive Prostate Cancer

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**14. ABSTRACT**

**Objectives:** In this application, we propose to build upon our current work to determine the association between fatty acid synthase (FAS) overexpression and intraprostatic fat as measured by in-vivo imaging using proton magnetic resonance spectroscopy imaging in the prediction of prostate disease aggressiveness. Mechanisms linking fatty acid synthase overexpression, lipid accumulation, lipid oxidation, and tumor aggressiveness will be explored using metabolomics. **Plan:** Employing a cross-sectional design we will recruit 50 men with low-grade and 50 men with high grade prostate cancer post-diagnosis as determined prior to prostatectomy. Each patient will complete one proton magnetic resonance spectroscopy imaging session and provide access to his prostatectomy tissue.

**Study aims**: Among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by Gleason scoring) we propose to: 1) Determine the correlation between FAS expression in prostatectomy samples and the amount of intraprostatic lipid using $^{1}$H magnetic resonance spectroscopic imaging (proton MRSI) with an endorectal coil. 2) Identify the association between FAS expression and FAS activity in prostatectomy samples, intraprostatic lipid as measured by MRSI and prostate tumor aggressiveness. 3) To quantify key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in the prostatectomy samples.

**15. SUBJECT TERMS :** none listed

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**INTRODUCTION:** Mounting evidence suggests that dysregulation of fatty acid synthase (FAS), the rate limiting multienzyme in the de novo formation of free fatty acids, is an early and important step in carcinogenesis and transformation to aggressive prostate cancer. Excess production of free fatty acids by FAS occurs through enhanced synthesis of malonyl-coA from acetyl-CoA and leads to increased cellular triglyceride formation and deposition. Thus we **hypothesize** that increased intraprostatic lipid concentration as measured by $^{1}$H Magnetic Resonance Spectroscopy (MRSI) will identify tissues with higher FAS activity, which in turn will be those that exhibit more aggressive disease. In more aggressive cancer tissues, we expect to find metabolic signatures of enhanced fatty acid oxidation. In showing an association between FAS protein overexpression by histology, in-vivo intraprostatic fat as measured by $^{1}$H MRSI, metabolic signatures of lipid oxidation and metabolism, and prostate cancer aggressiveness, our **objective** is to provide support for the novel application of this imaging modality for use in the clinical setting to determine the proper management of newly diagnosed prostate cancer. Specifically, among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by the 2011 National Comprehensive Cancer Network (NCCN) guidelines [6]) we propose to 1) determine the correlation between the amount of intraprostatic lipid using $_{1}$H magnetic resonance spectroscopic imaging (MRSI) with an endorectal coil obtained prior to prostatectomy with FAS protein expression measured in benign and cancer tissue from prostatectomy samples; 2) identify the association between FAS protein expression in prostatectomy samples, intraprostatic lipid as measured by $_{1}$H MRSI, and prostate tumor aggressiveness; and 3) quantify the association between key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in prostatectomy samples and FAS protein expression, intraprostatic lipid and tumor aggressiveness.

**BODY:** Department of Defense funding to allow initiation of this project was set up and received locally at the end of January/ beginning of February, 2013. From that point forward we have made progress in meeting the following items from our statement of work as described below (full SOW attached).

***Task 1.*** **Finalize clinical protocol and training (Shannon & Purnell) Months 1-6**

1. Develop tracking system for recording patient recruitment, contact and consent information; laboratory and specimen receipt and analysis. **(Shannon)**

*This task has been completed. All patient records are recorded using the Progeny system. Security is assured by maintaining all identifiers on the VA computer system with a crosswalk to a random unique ID maintained in the tracking program.*

2. Obtain IRB approval from Portland Veterans Affairs Medical Center (PVAMC) **(Shannon)** and Oregon Health & Science University (OHSU) **(Purnell)**

*This task has been completed. Portland VA Medical Center (PVAMC) IRB approval was received 9/9/2012. Oregon Health & Science University (OHSU) IRB approval was received on 12/28/2012.* ***2014 Update****: We initiated a move to the joint IRB (PVAMC+OHSU) in order to streamline all project modifications, assure we operated under the same protocol at all times as well as to minimize paperwork submission on 9/3/2013; our move was approved on 10/31/2013.*

3. Finalize and review services with Clinical and Translational Research Center (CTRC) bionutrition staff. **(Shannon & Purnell)**

*We are not utilizing the CTRC bionutrition staff at this time. We are, however, utilizing the CTRC core laboratory to process our urine and blood specimens since the first subject's enrollment onto this study on March 8th, 2013. This SOW point has been modified to reflect this change in our study plan.*

4. Arrange meetings between research coordinator and Advanced Imaging Research Center staff in order to: **(Purnell)**
    a. Identify point of contact cascade
    b. Collaboratively develop study-specific Standard Operating Procedures
    c. Train all staff on following research protocol exclusively
    d. Gather regulatory documents

*These tasks have been completed. Monthly research meetings are held with all study staff and the interdisciplinary investigational team. Point of contact has been identified for each step in the research process and an SOP has been developed for consistent recruitment of subjects and exchange of data from the urologist to the research coordinator to the MRSI technician and investigator to the pathologist (see Appendix 2; biopsy and MRSI measurement report form). Research protocol training has been completed with Ms. Farris and Mr. Stoller as well as all participating investigators. All regulatory documents are stored per VA protocol.* ***2014 Update****: As our clinical radiologist, Dr. Fergus Coakley, has emphasized, the accomplishment of assembling a multi-disciplinary team to meet on a monthly basis is of great import. We discuss the research process, its progress, provide quality improvement and care as well as maintain all aspects of the study as a cohesive group. Communication is clear, consistent and concise.*

*We have new, supporting coordination staff; one recruiting participants from OHSU (Ms. Martinez), another from PVAMC (Ms. Palma). The addition of staff has increased our capacity for assuring subjects have project staff with them/ in the vicinity for the research visit at all times. Our coordinating staff assures the post-procedure handout for complications is provided, and is a reliable escort through campus and the imaging facility back to familiar ground. Additional procedures have been identified, streamlined and instituted over the course of the past year both at our monthly meetings as well as with subgroups intimately involved with subjects. This includes such procedures as 1) enemas must be completed a full hour prior to research MRSI with endorectal probe, 2) scheduling each subjects' imaging research visit for a full 2 hours in order to accommodate multiple steps in support of image capture process, 3) taking pictures of the prostate at the time of pathology processing and 4) assuring clinical radiology interpretation not only goes back to the imaging investigators but to the respective urologic surgeon as well. All changes to procedure were reviewed and approved by the IRB prior to implementation.*

5. Review protocol and procedures with clinical staff; establish pathology residents' formal independent contracts **(Shannon)**

*Review of protocol and procedures has been an ongoing monthly task. Optimization of procedures has been ongoing and we have recruited 10 men whose data will only be utilized during this optimization time period. Independent contracts with the pathology residents occurred during the months of March and April 2013.* ***2014 Update****: Since our project's initial review and first DOD annual report, we have submitted 5 modifications and 1 continuing review. Please see 'Key Research Accomplishments' section for a full list and related descriptions.*

***Task 2.*** **Initiate subject recruitment and testing Months 6-30**

1. Identify potentially eligible patients, contact men and initiate recruitment **(Shannon)**
2. Complete consenting process and confirm eligibility for interested men **(Shannon)**
3. Conduct fasting blood collection, magnetic resonance spectroscopy imaging (MRSI) visits and prostatectomy tissue processing **(Purnell)**

*Progress towards completion of these tasks is ongoing; as of September 02, 2014, we have enrolled and consented a total of 38 men to the study. Since our last year's annual progress report, 30 men successfully completed an MRSI with no complications. We have one pending MRSI, one withdrawal and three screen fails (that is, no successful MRSI). We have collected specimens on 34 men; 2 prostatectomies are pending. As noted in our previous annual report we have added OHSU as a recruitment site and our average recruitment rate is 2 men per month. Based on this rate of recruitment we anticipate recruiting a minimum of 24 men over the next year.*

*The first ten test subjects' prostate tissue have been stained and reviewed and our clinical pathologist, Dr. George Thomas, continues to refine the process of determining percent stained. Also, the subcontract with Drs. Nagireddy Putluri and Arun Sreekumar at Baylor has been set up. Although we will be sending the first ten test subjects to them in the near future, the Baylor team requests that we batch samples to send in full at the end of the trial.*

*One of our newest internal procedures is to take photos of the prostate after the pathology residents have processed the prostates in order to further assist in the match-up between MRSI image voxels and the location from which the tissue is cut (**Figure 1**).*

*As proposed, we conducted an optimization study of our Magnetic Resonance Spectroscopic Imaging (MRSI) protocol. Specifically, two dimensional (2D) spectroscopy image sequence with fat and water suppression outside the imaging Volume of Interest (VOI) was implemented on the TIM Trio system. A total of eight saturation bands (six for fat and two for water) were used to minimize lipid/water contamination to the VOI. The 2D MRSI sequence details are: TR/TE: 1500 ms/30 ms; slice thickness: 5 mm; FOV 60 $cm^2$, 24 X 24 acquisition matrix, resulting in a 2.5 x 2.5 x 5 $mm^3$ voxel size. Each 2D MRSI acquisition lasts about 9.5 minutes and we acquire 3 MRSIs to cover prostate locations from apex to base, based on biopsy findings and radiologist's input (**Figure 2, a and b**). For internal reference and prostate segmentation purposes (**Figure 2c**), the same 2D spectroscopy image sequence was run on selected locations without water suppression. Due to the low matrix size (compared to standard MR imaging) adopted by spectroscopy imaging, signal localization becomes poorer and contamination from neighboring voxels can be significant. During the past year, we have developed and implemented a method to correct and clean up signal contamination (examples are **Figure 2, d and e**).*

**Figure 1:**

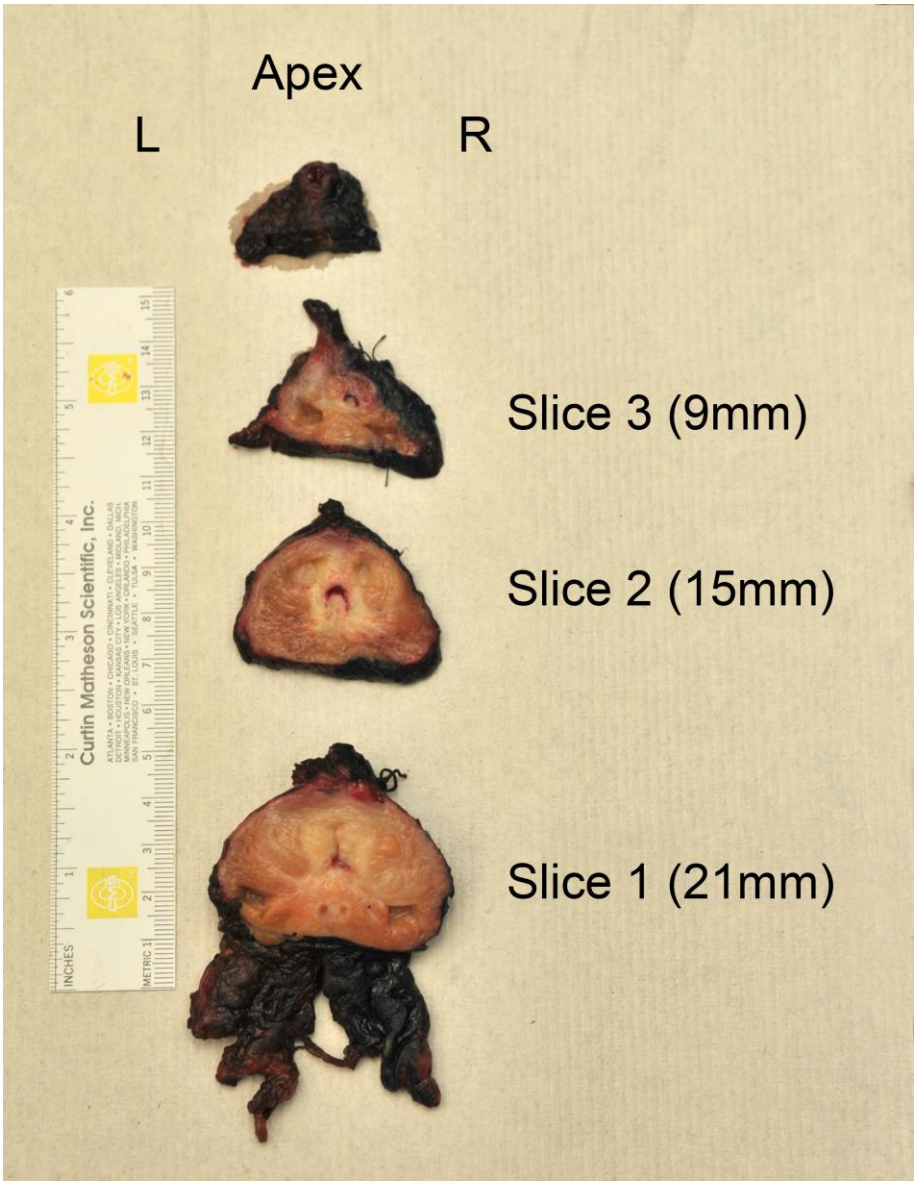
Apex
L
R
Slice 3 (9mm)
Slice 2 (15mm)
Slice 1 (21mm)
Curtin Matheson Scientific, Inc.
INCHES
METRIC

**Figure 2:**

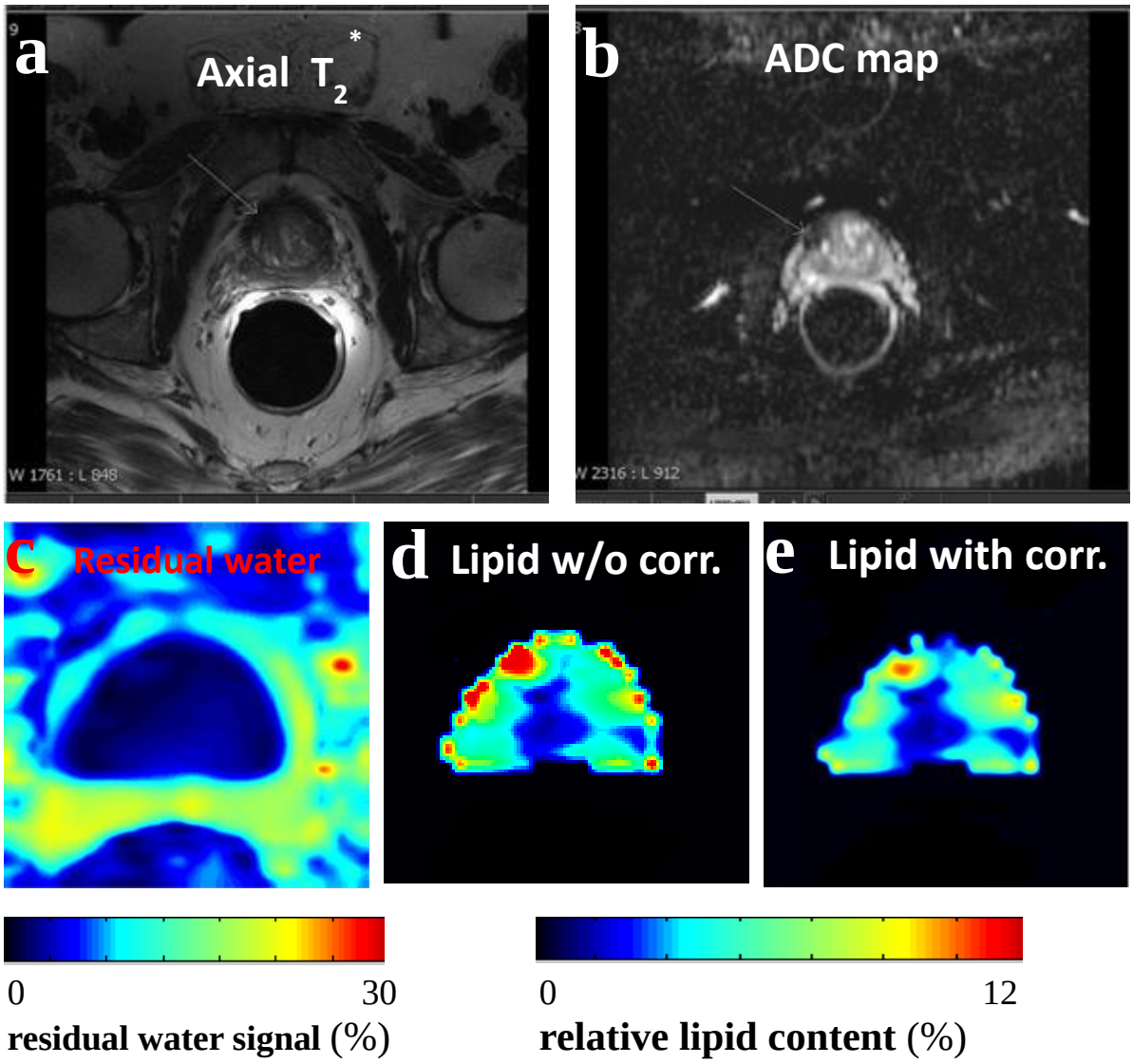


**Figure 2.** **a** and **b:** show an MRI visible lesion identified by the participating study Radiologist. **a**, $T_2$*-weighted axial image of a subject's pelvis area. **b,** the ADC (apparent diffusion coefficient) map of the same slice location. MRI visible lesion is indicated with white arrows in both panels. **c:** shows a residual water map zoomed to the prostate area. The generally dark blue area of the prostate provides sufficient contrast that leads to significantly simplified segmentation of the prostate. **d:** relative lipid content of the masked prostate cross-section uncorrected for signal contamination from fat contained in neighboring, extra-prostatic voxels. **e:** relative lipid content of the same data shown in **d** corrected for signal contamination from fat contained in neighboring, extra-prostatic voxels. The difference in patterns of lipid content between the uncorrected to the corrected heat maps underscores the importance of performing the correction in improving the specificity of the spectroscopy images.

4. Compensate men for their participation in study **(Shannon & Purnell)**

*Within a month of a man participating on our trial, they have either been compensated for a successful MRI and/or travel reimbursement.* ***2104 Update****; this task is ongoing and will continue until recruitment is complete.*

**KEY RESEARCH ACCOMPLISHMENTS:** Bulleted list of key research accomplishments emanating from this research.

**Year 01 – 2013**

- Portland VA Medical Center (PVAMC) IRB approval as of 9/9/2012.
- Standing monthly investigational team meeting initiated 11/8/2012.
- Added Medical Monitor, Arthur Hung, MD to project 11/14/2012.
- Oregon Health & Science University (OHSU) IRB approval as of 12/28/2012.
- Initiation of enrollment; first participant consented to study 2/22/2013.
- Continuing review PVAMC IRB approval 3/12/2013.
- Modification to add safety ocular x-ray to study; PVAMC IRB approval 3/29/2013.
- Modification to add safety ocular x-ray to study; OHSU IRB approval 4/28/2013.
- Dr. Fergus Coakley, OHSU Diagnostic Radiology Chair agrees to collaborate, consult and share his MRI in prostate cancer expertise with the investigational team, 5/20/2013.
- Modification to exclude recently-prescribed statin users (i.e.: on statin drug for less than 6 months) from study, increase to number of men (to 140); PVAMC IRB approval 6/18/2013.
- As of August 29, 2013, 9 men consented to study; 1 pending MRSI, 6 successful MRSIs, 2 screen fails.

**Year 02 - 2014 IRB and Research Updates**

- As of last year's report, we have enrolled 30 additional men to this study. Our veteran participants number 12, OHSU's are 18; please note that the first 10 subjects will not be included in any data analyses (the first 10 subjects acted as our 'test' subjects while we optimized our research project's multi-level, multi-resource, multi-system processes). We have a total of 24 successful (and analyzable) MRSIs, 1 pending MRSI, 3 screen fails and 1 withdrawal.
- Based on the optimization work, a standardized protocol for imaging, obtaining and processing tissue and obtaining and storing biologic specimens was put into place and recruitment into the primary study began 11/22/2013.

- All scientific investigators, study staff and clinical investigators continued to meet monthly to discuss study progress, necessary changes and review the timeline for study analyses.

- PVAMC and OHSU IRBs joined forces to provide researchers with one system for human subjects review and monitoring for projects that operate at both institutions. We submitted our modification to move the whole project to the 'joint' IRB as well as reconcile any remaining protocol differences; receiving JOINT IRB approval on 10/31/2013.

- Dr. Christopher Amling, OHSU Department of Urology Chair and surgical urologist agrees to collaborate and act as an addition recruitment site. Modification to add OHSU Department of Urology personnel (NP and Coord.) to the study; JOINT IRB approval on 11/7/2013.

- Due to confusion about the correct date to follow for continuing review, materials were submitted late for continuing review and the IRB approval lapsed for 3 weeks. All study activities were halted during this short time, regaining IRB approval on 1/21/2014.

- Dr. Mark Garzotto's conflict of interest form was incomplete and he was hence removed from the continuing review submission in order to rapidly re-gain IRB approval. Dr. Garzotto was re-added to the project on 3/24/2014.

- In order to communicate clearly and effectively with research participants, an enema instruction sheet was developed and approved by the JOINT IRB on 5/23/2014.

- At the time of the 2014 annual report update, a modification to add another OHSU Department of Urology clinician and update our post-MRI procedure handout is currently under JOINT IRB review.

**REPORTABLE OUTCOMES:** None to date

**CONCLUSION:** *As of the time of this progress report we have made great strides in developing the process and procedures necessary to effectively recruit patients into this study and carry out the MRSI so as to achieve our study aims. Close interaction between study staff, particularly the urologist, radiologist and pathologist has allowed us to work through the many details involved in successfully mapping regions of the prostate from an MRSI output to pathologic examination and tissue collection. We are now focusing on achieving our targeed patient recruitment goals and continue to meet regularly to discuss any subject difficulties and plan for analyses. Completion of this portion of our project has laid the groundwork for successfully addressing our aims of correlating intraprostatic lipid as identified by MRSI with FAS protein expression in areas of high lipid content, and with disease aggressiveness.*

**REFERENCES:** None to date

**ATTACHMENT:** Full Scope of Work

**Attachment 5: Statement of Work**
**Imaging prostatic lipids to distinguish aggressive prostate cancer**

***Task 1.*** **Finalize clinical protocol and training (Shannon & Purnell) Months 1-6**

1. Develop tracking system for recording patient recruitment, contact and consent information; laboratory and specimen receipt and analysis. **(Shannon)**
2. Obtain IRB approval from Portland Veterans Affairs Medical Center (PVAMC) **(Shannon)** and Oregon Health & Science University (OHSU) **(Purnell)**
3. Finalize and review services with Clinical and Translational Research Center (CTRC) bionutrition staff. **(Shannon & Purnell)**
4. Arrange meetings between research coordinator and Advanced Imaging Research Center staff in order to: **(Purnell)**
   a) Identify point of contact cascade
   b) Collaboratively develop study-specific Standard Operating Procedures
   c) Train all staff on following research protocol exclusively
   d) Gather regulatory documents
5. Review protocol and procedures with clinical staff; establish pathology residents' formal independent contracts **(Shannon)**

***Expected Products:***

Tracking system, IRB approval, supplies ordering, training.

***Task 2.*** **Initiate subject recruitment and testing Months 6-30**

1. Identify potentially eligible patients, contact men and initiate recruitment **(Shannon)**
2. Complete consenting process and confirm eligibility for interested men **(Shannon)**
3. Conduct fasting blood collection, magnetic resonance spectroscopy imaging (MRSI) visits and prostatectomy tissue processing **(Purnell)**
4. Compensate men for their participation in study **(Shannon & Purnell)**

***Expected Products:***

**25 Study Participants in Yrs 01 and 03.**MRSI measures, flash-frozen prostate tissue, stored blood specimens. **50 Study Participants in Yr 02.** MRSI measures, flash-frozen prostate tissue, stored blood specimens

***Task 3.*** **Data and Safety Monitoring (Shannon & Purnell) Month 12, 24, 36**

1. OHSU Knight Cancer Institute Data Safety Monitoring Committee (DSMC; responsible for conduct of cancer research) audits; review of all study related documents, assure full source documentation in place, make recommendations regarding each subject as well as continuation of the trial

***Expected Product:***

Completed DSMC report for submission to IRBs of record.

***Task 4.*** **Conduct immunohistochemistry analyses (Shannon) Months 12, 24, 32 (3 batches)**

1. Prostate specimens pulled from pathological archives for immunohistochemistry analyses. Expected N=25 batch 1, N=50 batch 2, N=25 batch 3
2. Utilize database for tracking specimen receipt and analysis

***Expected Product:***

Completed immunohistochemistry analyses

***Task 5.*** **Conduct metabolomics analyses (Purnell) Month 30-34**

1. Tissue specimens shipped to Dr. Sreekumar at Baylor Medical School for metabolomics analyses. Expected N=200
2. Utilize database for tracking specimen receipt and analysis

***Expected Product:***

Completed metabolomics analyses

***Task 6.*** **Final Analyses and Report Writing (Shannon & Purnell) Months 30-36**

1. Final statistical analysis of data from immunohistochemistry, MRSI and metabolomics measures will be performed
2. Prepare final report and initial manuscripts

***Expected Product:***

Completed and submitted final report to DOD

**Manuscripts**:

Primary findings –

Correlation of fatty acid synthase expression and intraprostatic lipids; measures of aggressive vs. indolent prostate cancer via proton magnetic resonance spectroscopic imaging

Association of fatty acid synthase expression and intraprostatic lipids in aggressive prostate cancer

Metabolomic quantification of fatty acid synthase expression and intraprostatic lipid accumulation in prostate cancer

Secondary findings –

Magnetic resonance spectroscopic imaging as a screening tool for aggressive vs. indolent prostate cancer