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TITLE: Imaging prostatic lipids to distinguish aggressive prostate cancer

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CONTRACTING ORGANIZATION: Oregon Health & Science University

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14. ABSTRACT <b>Objectives:</b> 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. <b>Plan:</b> 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.  <b>Study aims:</b> 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 <sup>1</sup> 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.					
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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 <sup>1</sup>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 <sup>1</sup>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 <sup>1</sup>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 <sup>1</sup>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.*

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

*We are not utilizing the CTCRC bionutrition staff at this time. We are, however, utilizing the CTCRC core laboratory to process our urine and blood specimens since the first subject's enrollment*

onto this study on March 8<sup>th</sup>, 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. 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.*

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.*

#### **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 August 28, 2013, 9 men consented to study; 1 pending MRSI, 6 successful MRSIs, 2 screen fails. We anticipate recruitment of 25 study participants after one full year of recruitment. Our first enrollment was in March 2013 and we are averaging approximately 1 new subject per month. This is slower than anticipated. To address this concern we are in the process of opening recruitment at OHSU as well.*

*As we had proposed, we have conducted optimization studies of our Magnetic Resonance Spectroscopic Imaging (MRSI) protocol in 5 men prior to formal enrollment. MR imaging slice was oriented according to pathologist's choice. The potential of using Flourinert as a susceptibility matching agent (1) for endorectal coil (Ecoil) imaging was also tested on phantoms (fat, water, and pork muscle piece) made in-house. Compared to Ecoil filled with air, slightly improved spectroscopic linewidth with Flourinert was only observed after manual shim was performed on the pork muscle phantom, mimicking in vivo prostate MRSI. Given that the linewidth of the water peak is already < 40 Hz after auto shim, the improvement is not expected to provide large enough gain to offset the additional time required for manual shim each time. Thus, 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<sup>2</sup>, 24 X 24 acquisition matrix, resulting in a 2.5 x 2.5 x 5 mm<sup>3</sup> 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 1).*

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.*

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

- 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.

**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. 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. 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.

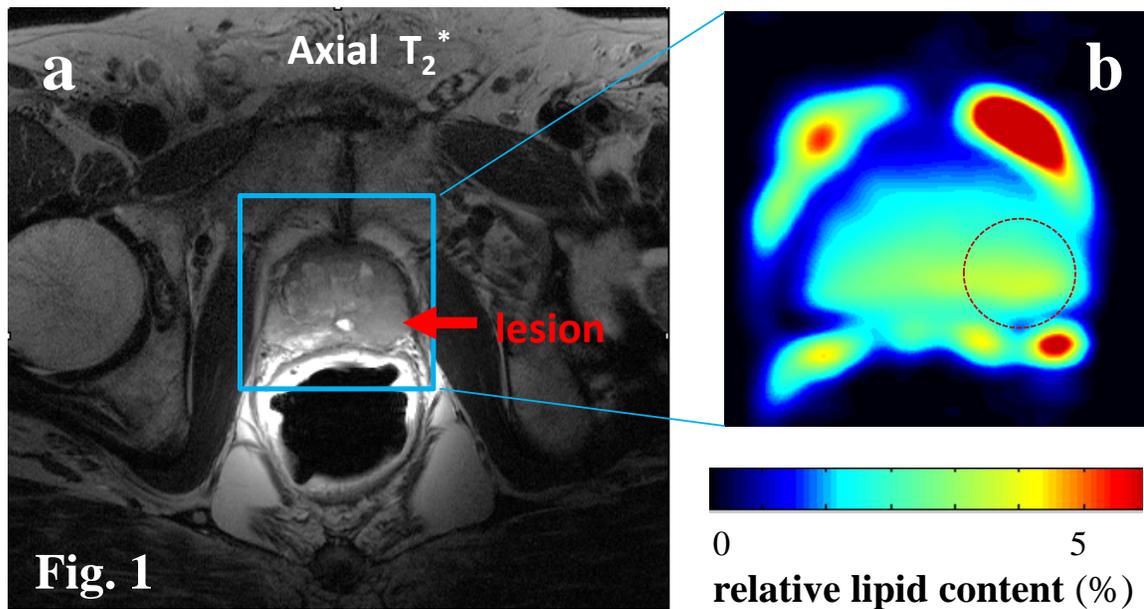
**REFERENCES:** List all references pertinent to the report using a standard journal format (i.e. format used in *Science*, *Military Medicine*, etc.).

1. Ocak I, Bernardo M, Metzger G, Barrett T, Pinto P, Albert PS, Choyke PL., Dynamic contrast-enhanced MRI of prostate cancer at 3 T: a study of pharmacokinetic parameters. *AJR Am J Roentgenol.* 2007 Oct; 189(4):849.
2. Scheenen TW, Klomp DW, Röhl SA, Fütterer JJ, Barentsz JO, Heerschap A. Fast acquisition-weighted three-dimensional proton MR spectroscopic imaging of the human prostate. *Magn Reson Med.* 2004 Jul;52(1):80-8.

**APPENDICES:**

1. Full Scope of Work
2. Combined prostate biopsy and MRI measurement report form (for pathologist use at the time of prostate tissue processing)

**SUPPORTING DATA:**



**Figure 1a** shows a T<sub>2</sub>\*-weighted axial image of a subject's pelvis area. A prostate lesion is indicated in red. Panel **1b** shows a zoomed lipid color map with the lipid content normalized to maximum total water and fat signal. Elevated lipid content is visible in the lesion area (red dashed circle). It is worth noting that periprostatic lipid content is generally high (2). This is obvious with noticeable hot spots around the prostate even when multi-band lipid saturation is prescribed.

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

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**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

## Imaging prostatic lipids to distinguish aggressive prostate cancer – REPORT FORM

Subject #VASI-XXX    MRSI Date: \_\_\_\_\_

Biopsy Pathology Report (Date: _____)	
LEFT SIDE (5 cores)	RIGHT SIDE (5 cores)
Left Base	Right Base
Left Upper Mid	Right Upper Mid
Left Lower Mid	Right Lower Mid
Left Mid Medial	Right Mid Medial
Left Apex	Right Apex

MRSI Prostate Image Measurements slice 1	
MRSI Date:	
Distance from apex	
Distance from urethra, if avail. (L/R)	
Distance from posterior margin	
Distance from left-right mid line (L/R)	
Distance from posterior margin (A-P)	
Largest cross-section measure ( $\Delta x$ by $\Delta y$ )	

MRSI Prostate Image Measurements slice 2	
MRSI Date:	
Distance from apex	
Distance from urethra, if avail. (L/R)	
Distance from posterior margin	
Distance from left-right mid line (L/R)	
Distance from posterior margin (A-P)	
Largest cross-section measure ( $\Delta x$ by $\Delta y$ )	

MRSI Prostate Image Measurements slice 3	
MRSI Date:	
Distance from apex	
Distance from urethra, if avail. (L/R)	
Distance from posterior margin	
Distance from left-right mid line (L/R)	
Distance from posterior margin (A-P)	
Largest cross-section measure ( $\Delta x$ by $\Delta y$ )	

