

Utility of Hyperpolarized ^{13}C -Pyruvate Metabolic Magnetic Resonance Imaging in the Diagnosis and Risk-Stratification of Prostate Cancer

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Metabolic Imaging in the Diagnosis and Risk-Stratification of Prostate Cancer Study

Protocol Synopsis

Study Objective	
Primary Objective:	To examine the safety and feasibility of performing hyperpolarized metabolic MRI in the diagnosis of prostate cancer.
Secondary Objectives:	To study the accuracy of hyperpolarized metabolic MRI to diagnose prostate cancer To examine the added utility of metabolic MRI over standard MRI imaging To perform correlative metabolic analysis related to cancer diagnosis. Using the research tissue when it is available.
Study Design	
Study Design:	This is a two-tiered pilot study in which there will be no randomization and no placebo treatment. This study will be to perform metabolic magnetic resonance imaging on men suspected to have a prostate cancer to understand if metabolic MRI can be safely performed on this population
Study Duration:	3 years
Enrollment Size:	6 patients
Number of Centers:	1
Inclusion Criteria:	1. Age greater than 40 and less than 80 years 2. Clinical suspicion or history of prostate cancer reflected by one of the following: - PSA > 4ng/ml - Abnormal DRE exam - Known tissue diagnosis of prostate cancer from prior workup 3. Patient planning to undergo either a MRI targeted biopsy or radical prostatectomy for prostate cancer workup or treatment
Exclusion Criteria:	1. Inability to undergo MRI scan 2. Inability to receive IV contrast as per institutional protocol.

Time frame:	3 months per patient
Expected Outcome:	We expect at the conclusion of this study to demonstrate the feasibility of performing metabolic MRI in a prostate cancer patient cohort.

1. INTRODUCTION AND STUDY RATIONALE

1.1 Introduction and Background

Recent advances in prostate cancer imaging have allowed for improved targeted prostate biopsy and more reliable diagnosis of high-risk prostate cancer. The study we propose will build off of these advances in an attempt to further improve our ability to non-invasively identify and precisely locate high-risk prostate cancer by using metabolic magnetic resonance imaging (MRI) of the prostate. The fact that metabolism in cancer is different than metabolism in normal tissue has been known for many years. This higher metabolic rate and turnover of nutrients such as glucose allows the cancer cell to rapidly divide and grow. Historically however, it was difficult to translate this knowledge that cancer has an altered metabolism into a practical application that impacted the clinical care of our patients. Recent developments in imaging technology, with the development of a process called hyperpolarized imaging, now makes it feasible to perform real-time MRI of metabolism as it differs within the body tissues (i.e. metabolic MRI). In this way, it is our hope that ultimately, by understanding the metabolic traits which are associated with aggressive prostate tumors, we will be able to perform targeted biopsies of metabolically active areas in the prostate and further improve our ability to even more reliably capture high-risk prostate tumors. The purpose of this study will be to perform metabolic magnetic resonance imaging on men suspected to harbor prostate cancer. It is ultimately our goal to understand if metabolic MRI can improve our ability to diagnose prostate cancer.

2. STUDY GOALS AND OBJECTIVES

2.1 Primary Objective:

- To examine the safety and feasibility of performing hyperpolarized metabolic MRI in the diagnosis of prostate cancer.

2.2 Secondary Objectives:

- To study the accuracy of hyperpolarized metabolic MRI to diagnose prostate cancer

- To examine the added utility of metabolic MRI over standard MRI imaging
- To perform correlative metabolic analysis related to cancer diagnosis. Using the research tissue when it is available.

2.3 Rationale for the Selection of Outcome Measures:

Prostate cancer is the second leading cause of cancer death in men; however, 88% of men with prostate cancer will die of other causes. This fact has fueled the current debate on balancing the benefits of treatment for aggressive prostate cancer with the harms of over-treating men who would have died of other causes.

Targeted MR/Ultrasound fusion-guided biopsy provides improved prostate cancer diagnostic accuracy and has highlighted the clinical

benefits of prostate cancer imaging advances [1]. **(Figure 1)**. We recently published in JAMA a large-scale study on the use of MRI-guided fusion biopsy for the diagnosis of prostate cancer which demonstrated a 30% increase in the diagnosis of high-risk disease [2]. However further improvement is needed as the sensitivity for identification of intermediate to high-risk prostate cancer is still only 77% [2]. One limitation is that MP-MRI looks mainly at prostate anatomy such as tissue density, water content, and vasculature to determine cancer risk. Emerging research has turned to functional cues such as local gene expression and metabolic activity to improve our ability to identify high-risk disease [3]. Indeed, our group recently demonstrated that gene expression profiling can increase predictive ability to identify extracapsular extension (i.e., stage T3 prostate cancer) from an area under the curve (AUC) of 0.73 for imaging alone to an AUC of 0.85 ($p < 0.001$, unpublished data). Our group is actively studying how metabolic profiling can similarly be integrated into becoming a clinically meaningful risk-stratification and diagnostic tool.

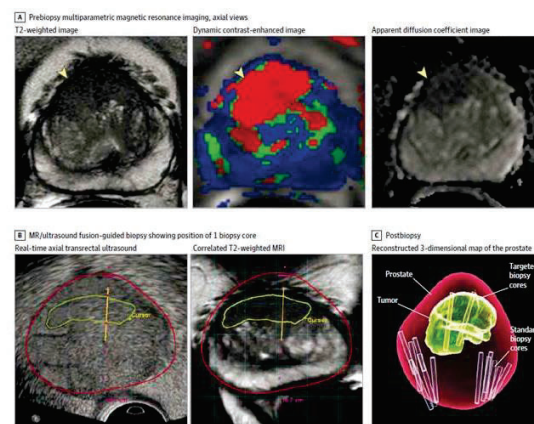


Figure 1: Schematic of MR/US fusion biopsy process. a: A prebiopsy MRI is performed which demonstrates a lesion seen on multiple sequences concerning for cancer (yellow arrow). b: On the day of MR/US fusion guided biopsy, real-time transrectal ultrasound is fused with the pre-biopsy MRI data. The lesion is not visible on ultrasound, however the MRI data is useable to guide the location of the biopsy. c: The final results of the biopsy demonstrate the standard 12-core biopsy missed sampling the anterior located lesion, however the targeted biopsies obtained nice samples of the lesion which was ultimately found to be high-grade (Gleason 9) prostate cancer. Image adopted from Siddiqui MM et al. "Comparison of MR/Ultrasound Fusion-Guided Biopsy With Ultrasound-Guided Biopsy for the Diagnosis of Prostate Cancer" JAMA 2015; 313(4): 390-397

The prostate is a particularly interesting gland from a metabolic perspective. In most of the body, glucose is metabolized through glycolysis into pyruvate which then enters into the citric acid cycle and is converted to carbon dioxide and ATP. In the normal prostate, by contrast, the citric acid cycle enzyme *aconitase* is inhibited by zinc and there is, as a consequence, a build-up of citrate, the substrate for *aconitase* [4] (**Figure 2**). Malignant prostate cells lose this ability to inhibit *aconitase* leading to lower citrate levels.

MR Spectroscopy Imaging (MRSI) uses this principle of prostate citrate metabolism to identify areas of potential tumor. In diagnosis of prostate cancer, suspicious regions can be identified as low levels of citrate. However, inflammation can also consume citrate locally and lead to a false positive which limits the modern-day utility of MRSI [5-8]. Even the combination

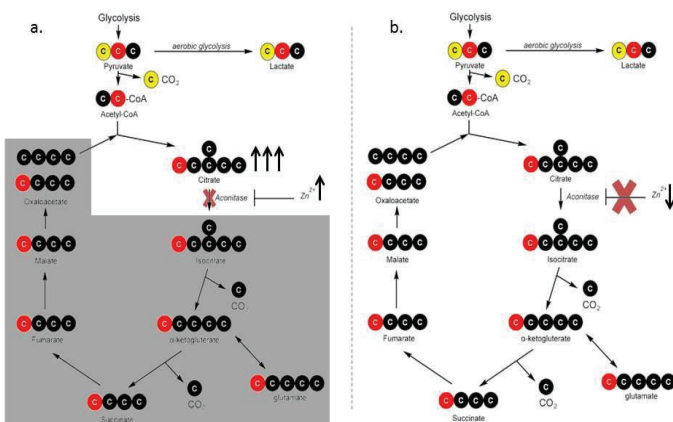


Figure 2: Alterations in the citric acid cycle between prostate cancer and benign prostate: Yellow carbon is the ^{13}C labeled carbon on pyruvate. **a.** Metabolism in benign prostate demonstrating increased zinc, inhibition of the citric acid cycle and increased citrate. **b.** Prostate cancer reverses inhibition of aconitase and decreases buildup of zinc.

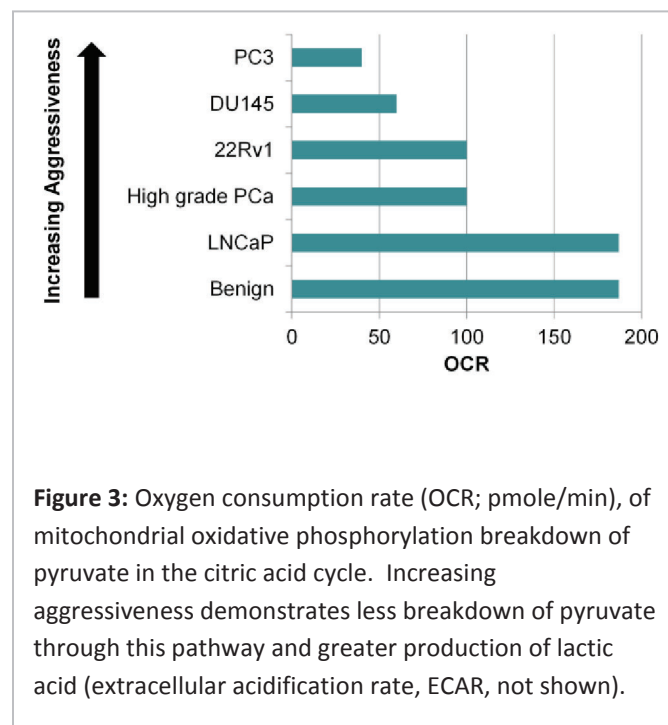


Figure 3: Oxygen consumption rate (OCR; pmole/min), of mitochondrial oxidative phosphorylation breakdown of pyruvate in the citric acid cycle. Increasing aggressiveness demonstrates less breakdown of pyruvate through this pathway and greater production of lactic acid (extracellular acidification rate, ECAR, not shown).

of the MRI/MRS techniques provided only limited improvement over transrectal ultrasonography [9-11].

In cell lines, the relationship of pyruvate breakdown in the citric acid cycle via aerobic oxidative phosphorylation versus lactic acid via anaerobic breakdown has been studied by our group in prostate cancer cell lines of varying aggressiveness (**Figure 3**). We have demonstrated that cell lines of increasing metastatic potential indeed demonstrate a tendency to not break down pyruvate via citric acid cycle and instead generate lactic acid instead. **We have also now generated preliminary data demonstrating uptake of ^{13}C labeled compounds in human tissue processed *ex vivo* and that differences of metabolism of labeled compounds such as pyruvate, glucose, and methionine are detectable between tumor and benign tissue.** For this

reason, we have increasing confidence that the next step, measuring tumor metabolism *in vivo* in humans will yield promising results to identify and risk stratify tumors by aggressiveness.

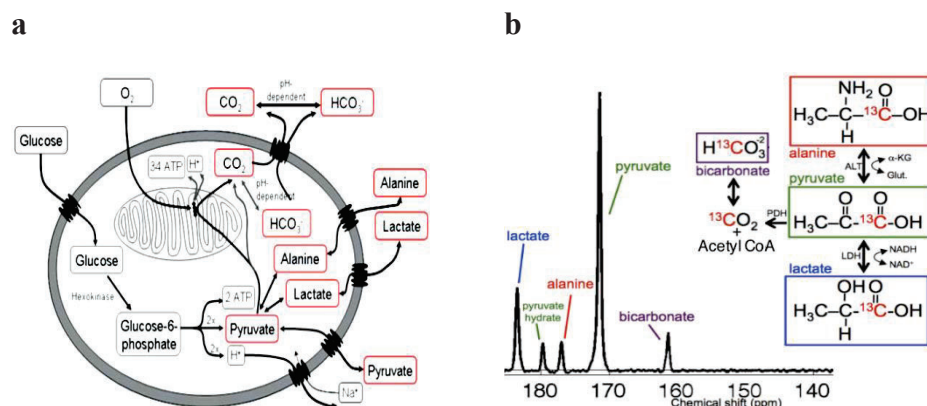
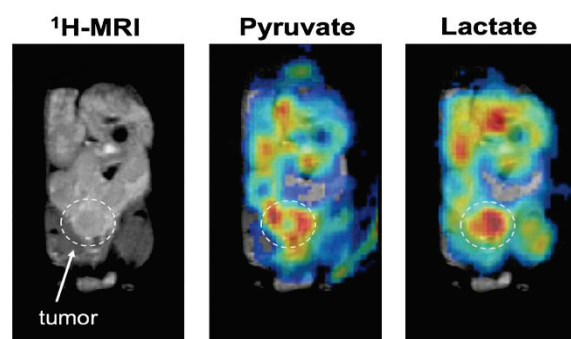


Figure 4 Pyruvate metabolism.

(a) Metabolic products detectable with $[1-^{13}\text{C}]\text{Pyr}$ are shown in **red**. **(b)** Single acquisition *in vivo* ^{13}C spectrum from a rat heart following the injection of HP $[1-^{13}\text{C}]\text{Pyr}$. Pyr and its products $[1-^{13}\text{C}]\text{Lac}$, $[1-^{13}\text{C}]\text{Ala}$, and $^{13}\text{C}\text{-Bic}$ are easily visualized. A small fraction of the $[1-^{13}\text{C}]\text{Pyr}$ is in equilibrium with metabolically inactive $[1-^{13}\text{C}]\text{Pyr-hydrate}$. Figure 4a from Golman et al., Acad. Rad. 13(8), 2006.

The recent advent of HP ^{13}C MRS [12], which achieves dramatically enhanced signal-to-noise ratios (SNRs) using a process known as dynamic nuclear polarization, provides unprecedented opportunities for **real-time imaging of *in vivo* metabolic pathways critical to the identification and evaluation of cancer** (see [13] and references therein). To date, the leading *in vivo* HP metabolic imaging candidate is $[1-^{13}\text{C}]\text{pyruvate}$. Pyruvate is an endogenous substance that plays a central role in both catabolic and anabolic metabolism with measurements of the downstream metabolic products of lactate, alanine, and bicarbonate reflecting the activity of the enzymes and coenzymes catalyzing the respective reactions (**Figure 4**).



It has been shown that metabolism of HP pyruvate is sensitive to the high glycolytic rates in tumors [14, 15]. **Figure 5** shows our

preliminary data from a transgenic adenocarcinoma of mouse prostate (TRAMP) mouse after injection of HP [1-¹³C]pyruvate showing higher conversion to lactate in the tumor. Furthermore, lactate production is correlated with tumor grade in TRAMP models [14], and [1-¹³C]pyruvate was the substrate of choice for the first clinical trial of *in vivo* HP MRS in prostate cancer patients [16], demonstrating the translational relevance of this technology.

Figure 5 Metabolic maps of Pyruvate and Lactate from a TRAMP mouse after injection of 80-mM HP [1-¹³C]Pyr.

3. INVESTIGATIONAL AGENT

3.1 Preclinical Data

A pre-clinical program has been performed in which the safety of both [1-¹³C]pyruvate and the electron paramagnetic agent (EPA) AH111501 (the 2 novel components in Hyperpolarized [1-¹³C]Pyruvate Injection) has been demonstrated. The program included expanded acute-dose studies in Sprague-Dawley rats from which the No-Observed-Adverse-Effect-Levels (NOAEL) were estimated as 1784 mg/kg body weight (bw) for pyruvate and 34 mg/kg bw EPA for the

500 mM formulation of pyruvate and 892 mg/kg bw for pyruvate and 17 mg/kg bw for EPA for the 250 mM formulation (the lower NOAELs merely reflect the fact that lower doses of the 250 mM formulation were given as maximum dosing volumes were limited to 20 ml/kg bw for rats for both formulations) (Investigators' Brochure). The 500 mM pyruvate formulation was also tested in an expanded acute-dose study in beagle dogs; the NOAEL for pyruvate was 446 mg/kg bw and 8.5 mg/kg bw for EPA. In addition, the effects of pyruvate and EPA have been studied in local tolerance studies, genotoxicity studies, and central nervous system studies. The cardiovascular effects of formulations of pyruvate were studied in conscious and pentobarbital/fentanyl-anaesthetized dogs. The conscious dog was considered the most relevant model for extrapolating results to healthy human volunteers and the anaesthetized dog as a sensitive model for extrapolating results to subjects with a compromised baroreflex function (as may be the case in elderly subjects with prostate cancer, which is one of the target populations in which use of Pyruvate Injection is planned).

Based on the cardiovascular effects (vasodilatation leading to small, short-lasting, but reproducible reductions in blood pressure and compensatory increases in heart rate) observed in the most sensitive model, the NOAEL for Pyruvate Injection is 1.4 ml/kg bw (or 100 ml for a 70 kg subject) of a 250 mM formulation (equal to 31 mg/kg bw of pyruvate). A 250 mM formulation will be used in the study described in this protocol.

Canine imaging studies have been performed at UCSF. The objective of these was to perform experiments that would evaluate the feasibility of observing metabolic changes using low doses of a 250 mM formulation of Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate Injection in a dog model. These studies were designed to provide a realistic test of the methodology that will be applied in exploratory imaging studies in humans, as the same coil geometry and data acquisition parameters were used for the dogs as are being planned for humans. Specific goals of the study were, therefore, to test the improved radiofrequency coils and pulse sequences being developed for low-dose applications, to determine the dynamics of arrival of the $[1-^{13}\text{C}]$ pyruvate bolus, to evaluate the signal to noise ratio (SNR) of $[1-^{13}\text{C}]$ pyruvate in healthy dog prostate, and to investigate whether its metabolic products could be observed at dose levels of one-quarter (0.36 ml/kg) and one-eighth (0.18 ml/kg) of the dose previously demonstrated to be efficacious in the healthy dog (1.4 ml/kg).

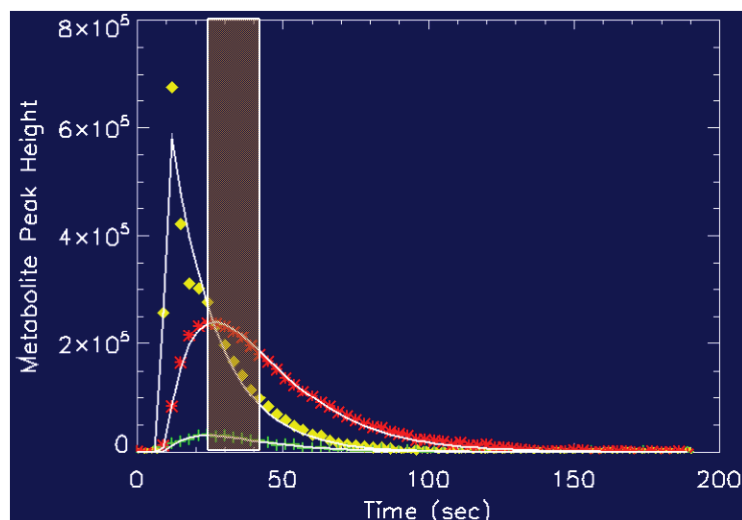


Figure 6 Dynamics of delivery and metabolism of hyperpolarized $[1-^{13}\text{C}]$ pyruvate in the canine demonstrating an optimum window to maximize signal-to-noise (SNR) ratio (shaded brown area) for 3-D ^{13}C spectroscopic imaging (yellow = pyruvate, red = lactate, green = alanine). The optimum window to maximize SNR for lactate occurs during a time when the lactate SNR is relatively constant due to a balance between its synthesis from pyruvate and its T1 decay.

The canine studies demonstrated that high spatial and temporal resolution anatomic and hyperpolarized $[1-^{13}\text{C}]$ pyruvate images could be acquired from the prostate using the coils and fast ^{13}C magnetic resonance spectroscopic imaging (MRSI) sequences designed for human studies (Figure 6 and Figure 7). From the dynamic data that were acquired it was determined that an imaging window could be selected based on the kinetics of delivery and metabolism of

the hyperpolarized $[1-^{13}\text{C}]$ pyruvate (Figure 6) that will maximize SNR for the MR signal originating from the $[1-^{13}\text{C}]$ lactate metabolite. The obtained SNR levels with two different imaging sequences are plotted in Figure 7. The conclusion of the canine studies was that images with a high lactate SNR can be assumed to be obtained also in the human prostate with Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate Injection in the dose range being proposed in the present study.

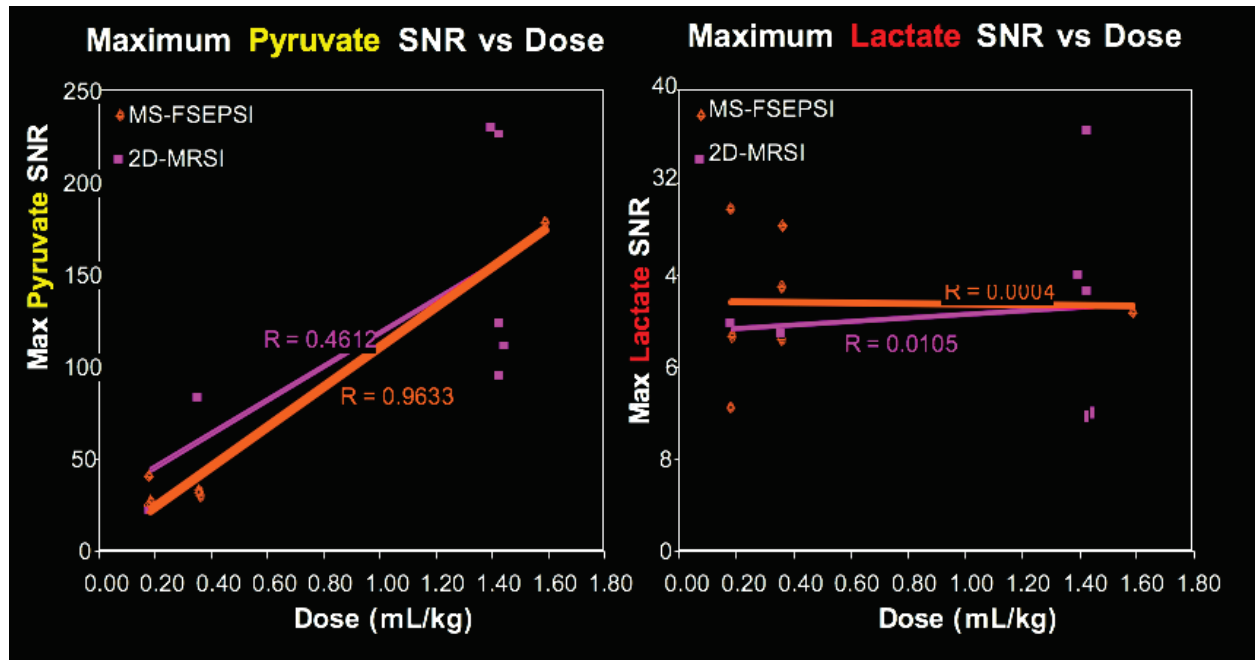


Figure 7 Hyperpolarized $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate SNR versus dose of Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate Injection for 3-D fast ^{13}C MRSI techniques. The curves represent 2 different pulse sequences that give similar results.

Conclusions from pre-clinical experience

Based on the overall results, it was expected that doses of Pyruvate Injection up to 1.4 ml/kg bw would be well tolerated. However, data from the pre-clinical studies in conscious and anaesthetized dogs indicated that attention should be paid in initial human studies to the possibility of mild cardiovascular effects occurring soon after injection, similar to those that were reported when large volumes of Pyruvate Injection were administered rapidly to dogs (i.e., vasodilatation leading to small, short-lasting but reproducible reductions in blood pressure and compensatory increases in heart rate).

3.2 Dose Rationale

Two phase 1 safety and tolerability studies in which Pyruvate Injection, produced from [1-^{nat}C]pyruvate, or placebo was injected into 36 young healthy volunteers and 30 elderly (60 years) volunteers (Investigators' Brochure) have provided an indication of the range of doses that are well tolerated by these subjects (up to 0.43 ml/kg bw). A dose of 0.43 ml/kg bw was also used and well tolerated in a phase 1 safety and exploratory imaging study of performing MRSI of [1-¹³C]pyruvate in 31 patients (median age was 63 years, range 45 to 75) with biopsy-proven prostate cancer and intact prostates [16]. The study demonstrated the feasibility of capturing regions of increased HP pyruvate-to-lactate conversion in high-grade versus low-grade cancer versus benign tissue.

4. STUDY DESIGN

4.1 Overview of Study Design

This study is designed as a two-stage pilot study in patients who are suspected or known to have prostate cancer to establish a process of performing metabolic MRI on this population. Ultimately this will lay the foundation to study if metabolic MRI can improve our ability to diagnose prostate cancer. The overall design of this study will be to initially recruit 10 patients in a 12-month period to fine tune the technical aspects and calibration of generating optimal scanning protocol followed by an accrual of 26 patients over a ~24 month period to assess the reliability of this scanning protocol. We will recruit from men who have opted for radical prostatectomy as the approach of choice for management of their prostate cancer or who are highly suspected of prostate cancer and are planned to undergo a MRI-US fusion targeted prostate biopsy. The candidate pool should be approximately 60 men a year of which we hope ~20% will be eligible and agreeable to this study. Men who are likely to have MR-visible lesions will be selected for this pilot study cohort.

For eligibility criteria, we will select patients who are suspected of prostate cancer due to elevated PSA or abnormal digital rectal exam OR who have a known prostate tumor. We will recruit men who are already planning (independent of the trial) to undergo some intervention for diagnosis or treatment of the possible prostate cancer. Men unable to undergo an MRI (pacemaker, claustrophobia, metal in their body, inability to receive IV contrast as per institutional protocol) will be excluded from the study.

These men who have already decided upon prostate biopsy or radical prostatectomy will have the metabolic MRI performed within a 3-month period prior to the procedure. Men entering this study will be consented for research tissue donation either as additional biopsy cores from lesions sampled as well as normal appearing areas of the prostate in patients undergoing fusion biopsy, or from the prostate specimen in men undergoing radical prostatectomy. Blood and urine samples for research correlative analysis will also be collected at the time of procedure.

Clinical ^{13}C imaging: For clinical ^{13}C prostate imaging we will use CMIT's GE 750w 3T MRI scanner and follow same radiofrequency coil setup as described by Nelson et al. [16]. A GE dual-tuned $^1\text{H}/^{13}\text{C}$ receive-only endorectal coil will be used for signal reception while signal excitation will be performed with a ^{13}C clamshell coil and the scanner's ^1H body coil. For dynamic metabolic imaging we will apply a fast chemical shift imaging (CSI) sequence with a targeted nominal isotropic resolution of approximately 5 mm. Immediately after the ^{13}C imaging, the residual hyperpolarized magnetization will be used to acquire MR spectroscopic data using a simple pulse-and-acquire pulse sequence. With respect to dose (0.43 ml/kg bw) and properties of the HP pyruvate, we will use the parameters described in the safety study by Nelson et al. [16] as they were well tolerated with no adverse events of concern. Dynamic ^{13}C imaging will be initiated at the end of the injection. The metabolic imaging will be complemented with the standard clinical ^1H MRI including contrast-enhanced T1-weighted and T2-weighted imaging as well as diffusion-weighted imaging.

During the initial 10 patient stage of this pilot study, our team will focus on optimizing a few key processes to successfully perform the metabolic MRI scans. These include optimization of imaging parameters such as excitation flip angle and temporal and spatial resolution as well as assessment of image artifacts.

After the clinical process and calibrations are optimized, we will progress to the validation stage of this pilot study in which these settings will be applied to 26 consecutive patients. A successful scan will be determined by achievement of a minimal signal to noise ratio (SNR) of the ^{13}C -pyruvate signal and corresponding product signals. Based on limited published literature on this topic, we have identified that a SNR of at least 3 at one of the time points or a total SNR (as data summed over time points) of at least 36 are reasonable criteria for a successful scan (17).

^{13}C Imaging Data Processing: The ^{13}C data will be reconstructed to generate time-resolved metabolic maps of pyruvate, lactate, alanine, and bicarbonate. Afterwards the maps of corresponding apparent rate constants k_{PL} , k_{PA} , and k_{PB} or equivalent parameters will be calculated.

MRI/US fusion prostate biopsy: MRI/US fusion biopsy will be performed using established methods and clinical processes with the UroNav fusion prostate biopsy platform. With this process, the MRI is reviewed per standard clinical practice by the radiologist and areas of concern are outlined using a DynaCAD workstation located in the radiology reading room which is used to segment the prostate and areas of concern. This segmentation then is transferred to a biopsy fusion platform which overlays the real-time trans-rectal ultrasound images with the processed MRI images with lesion of concern outlined. The lesion is thus overlaid on the ultrasound images which can then be targeted with the biopsy needle and sampled. If a metabolic hotspot is found to overlap a target lesion on MP-MRI, the lesion will

simply be target biopsied based on the target lesion and the information regarding the metabolic hotspot will be inferred from the target lesion biopsy.

Pathology: Pathology will be compared from the region of interest demonstrated on metabolic MRI and the correlating pathologic specimen obtained from radical prostatectomy or MR/US fusion biopsy.

Metabolomic analysis: Research tissue samples will be collected and flash frozen to preserve metabolic milieu. We will use a gas chromatography (GC) and liquid chromatography (LC) based high-resolution untargeted mass spectrometry approach for global interrogation of relative abundance of endogenous metabolites. The samples will be resolved on H-class UPLC systems and injected on the QTOF (Xevo G2-S). Metabolites with a p-value less than 0.05 will be identified. We will then select the most significant metabolites using a penalized selection method that corrects for overfitting (feature selection for Generalized Linear Mixed Models by L1-penalized estimation). For LC-MS based metabolomics data, ions will be identified through mass-based searches in four databases: Metlin, Madison Metabolomics Consortium Database, Human Metabolome Database, LIPID MAPS.

4.2 Randomization

This is a non-randomized, single arm pilot research study.

4.3 Subject Selection

4.3.1 Inclusion Criteria

1. Age greater than 40 and less than 80 years
2. Clinical suspicion or history of prostate cancer reflected by one of the following:
 - PSA > 4ng/ml
 - Abnormal DRE exam
 - Known tissue diagnosis of prostate cancer from prior workup
3. Patient already planning to undergo either a MRI targeted biopsy or radical prostatectomy for prostate cancer workup or treatment

4.3.2 Exclusion Criteria

Inability to undergo MRI scan

Inability to receive IV contrast as per institutional protocol

4.3.3 Number of Participants

Targeted enrollment is 6 patients with complete 3-month follow-up evaluations.

4.4 Subject Recruitment Plans

Potential study participants will be recruited from the Urology division at the University of Maryland Medical Center. The Principal Investigator and/or research team members will identify patients whose medical history is consistent with inclusion criteria and inform them that they may be eligible to participate in the study.

4.5 Consent Process

Patients being considered for enrollment in the trial will be administered informed consent for study participation. Informed consent for study participation must be obtained prior to the procedure. Written informed consent must be recorded appropriately by means of participants' dated signatures.

It is the responsibility of the Principal Investigator or his designated representatives to give each participant, prior to inclusion in the study, full and adequate verbal and written information about the objectives and the procedures of the study and the possible risks involved. The participants must be informed about their right to withdraw from the study at any time and for any reason without sanction, penalty, or loss of benefits to which they are otherwise entitled and that withdrawal from the study will not jeopardize their future medical care. Furthermore, it is the responsibility of the Principal Investigator or his designated representatives to obtain a signed current version of the approved informed consent form from each participant prior to performing any study-related procedures. The participant must receive a copy of the signed and dated informed consent form.

Patients should not be coerced, persuaded, or unduly influenced to participate or continue to participate in the study. Potential participants must be given ample time and opportunity to inquire about details of the study and all questions about the study should be answered to the satisfaction of the patient.

4.6 Early Withdrawal of Subjects

Every effort will be made to follow the progress and monitor the safety of patients who hyperpolarized metabolic MRI. If a patient does not return for a scheduled visit, attempts to contact the patient will be made and documented in source medical records and the electronic case report form (CRF).

If a participant chooses to withdraw from the study, the reason(s) for withdrawal will be recorded. If more than one reason is given, the primary reason will be identified. If possible, a complete, final examination will be performed on all patients who withdraw from the study.

5. STUDY PROCEDURES

This is a single center, prospective, non-randomized study. Patients whose medical history is consistent with high risk prostate cancer will be screened for potential eligibility

5.1 Study Schedule

Study Schedule				
Assessments	Screening Baseline	MRI	7±3-day follow up	Intervention (within 3 months of MRI)
Written Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Demographics	X			
Lab test	X			
Vital Sign Monitoring		Pre and Post Injection		
Metabolic MP-MRI Prostate		X		
Prostate Biopsy OR Prostatectomy				X
Research specimen		X		X
Adverse Event	X	X	X	X

5.2 Screening for Eligibility

Patients will be screened for eligibility upon presentation to the clinic. This can occur up to 60 days from or on the day of the Procedure/Baseline. Patients will undergo routine history and physical examination. Patients who are suspected of prostate cancer due to elevated PSA or abnormal digital rectal exam and whose medical history is consistent with inclusion criteria and inform them that they may be eligible to participate in the study. Then we will ask them to sign the study informed consent form. A creatinine blood test to determine eGFR for suitability for MRI contrast and study eligibility will also be drawn during this period.

5.3 Procedure

The patient subject in consultation with his physician (Dr. Siddiqui) opts for either an MRI-targeted biopsy or radical prostatectomy for prostate cancer workup or treatment. At this point the patient will be informed of this study. The patient will inform Dr. Siddiqui about his willingness to participate in the study. The HP ¹³C imaging procedure will be performed during a regular MRI imaging session scheduled for the patient that includes including contrast-enhanced T1-weighted and T2-weighted imaging as well as diffusion-weighted imaging. At the beginning

of the imaging session a special endorectal coil will be inserted that is necessary to detect the metabolic signal. After performing ^1H imaging to locate the prostate the ^{13}C imaging sequence will be prescribed. After injection of the HP ^{13}C -pyruvate the ^{13}C imaging will be started. Following the ^{13}C imaging and removal of the endorectal coil the standard clinical ^1H MRI will be completed.

Subjects will receive the HP ^{13}C -pyruvate under direct supervision of study personnel. The administration volume will be checked and the kit component vial codes and volume administered will be recorded in each subject's CRF. The dose will be calculated based on the subject weight with 0.43ml per Kg of body weight (0.43ml/kg BW). The maximum volume to be administered will be 42 mL. For patients with a weight higher than 95 Kg, we will reduce the administered dose according to the produced amount. The administered amount will be recorded on the study CRF form.

Injection site monitoring, blood pressure and heart rate vital sign measurements will be performed immediately before injection and at 5 and 30 minutes and 2 hours after injection. Abnormal injection site findings include, but are not limited to, extravasation, bleeding, hematoma, redness, and infection.

5.4 Follow up

All patients will have phone calls as part of standard clinical care by clinical staff post procedures to check for issues or concerns. Patients will also have regularly scheduled clinical follow-up as appropriate for their diagnosis and workup results.

6. RISKS

The following risks are associated with this study:

Phlebotomy: Minor risks associated with phlebotomy include pain, bruising, feeling faint or rarely, brief loss of consciousness or infection at the site of venipuncture. To reduce these risks, experienced individuals will perform phlebotomy with the subject comfortably seated or lying down. Blood for the study will be drawn during the same time that blood is being drawn for routine clinical care when possible.

Prostate tissue sample collection: Prostatectomy is performed as standard care. Samples of prostate tissue will be collected from residual tissue in the pathology laboratory. There is no risk related to collection of residual tissue.

MRI: MRI has no ionizing radiation and is safe when used on subjects who are appropriately screened for safety contraindications. Subjects with any contraindications for a MRI scan, or who have claustrophobia, will be excluded from the study. These contraindications will be assessed on the initial eligibility interview. They will be reassessed before the MRI is obtained, and any person with a contraindication at that time will be excluded from the study. The MRI scanner makes a loud knocking sound. The knocking sound depends on the type of scan. Participants will be given ear protection for the scan. Some of the radio frequency imaging coils, imaging software and devices being used in your scan are not approved by the FDA but are similar to counterparts that have been approved by the FDA (GE Healthcare Coils test report in Appendix).

Risk of Intravenous Gadolinium based Contrast agents:

The gadolinium-based contrast injection is being given to patients as part of their routine clinical care.

- 1) The injection of contrast does not usually cause pain, but 1 in 100 people may notice discomfort, tingling, or warmth in the lips, metallic taste in the mouth, tingling in the arm, nausea, or headache that lasts for a few minutes. Insertion of the catheter may also cause minor pain, bruising, and/or infection at the injection site.
- 2) There is a very rare risk of an allergic reaction to gadolinium. However, a severe allergic reaction occurs in less than one in 300,000 people.
- 3) People with severe kidney failure who receive gadolinium are at risk of developing Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD). This disease causes fibrosis (the formation of too much connective tissue in the skin and internal organs). This is a serious disease, which can result in death.

The patient should notify the study team or MRI staff if they are known to be allergic to gadolinium or have kidney problems

MRI-guided prostate biopsies will be done as standard of care. At the time of MRI-guided prostate biopsy, 1 additional biopsy sample will be collected for research purposes from each lesion biopsied. Typically during standard care prostate biopsy procedures, approximately 14-17 biopsy samples are routinely collected. Collection of one additional biopsy sample for research purposes does not present additional risk for the participant over that of the standard care biopsy procedure. Depending on the number of lesions present, no more than 2-6 additional tissue samples will be collected per patient. The risk of additional biopsies is minimal as it is not unusual using other paradigms of prostate biopsy to perform 24 biopsy samples of the prostate. The prostate is anesthetized with local nerve block prior to prostate biopsy such that the additional biopsies should not lead to any additional pain either for the patients. Theoretically, there is a risk of minor bleeding or infection with the additional biopsy samples. However, there is no evidence that 2-6 additional biopsies result in any greater risk for bleeding and infection than the standard number of biopsy samples. The clinical biopsies will be obtained first so that the research biopsies do not interfere with obtaining the clinical biopsies. In cases where the patient is uncomfortable and wishes not to obtain research biopsies even after prior consent, research biopsies will not be procured. Tissue will only be procured if it is certain that such procurement has an absolute minimal risk of interfering with clinical care in the patient. Accordingly, even if the patient has consented to tissue procurement, if there is any concern of insufficient tissue for clinical care, no tissue will be procured in any situation where it is thought clinical care may be compromised.

Risk of Pyruvate Injection: There are no known risks from injection of Pyruvate. However, it is possible that the subject may react to Pyruvate injection such as flushing, feeling hot, dysgeusia (a taste disorder that causes foul, rancid, metallic, or salty taste perception), and dizziness.

Unknown Risks: There may be risks or side effects related to the injection of pyruvate that are unknown at this time. The subject will be told if any significant new findings become known that may affect your choice to remain in the study.

Loss of Confidentiality: In the unlikely event that confidentiality is breached, participants may experience negative emotions (e.g. anger, anxiety, embarrassment, worry). There is a risk that an unauthorized individual or entity may obtain and use a participant's health information or research results (including genetic tests) inappropriately. To minimize the risk of loss of confidentiality, study files containing identifying information (e.g. consent form, contact information) will be stored in locked cabinets in the offices of the PI or research coordinators. Participants will be assigned a unique Study ID number which will be used to identify samples, research data and health information. Data will be stored in a controlled-access, database. Access to the study identification codes or other personally identifiable information will be restricted to the PI, essential study team members and, upon written request, to the IRB or other appropriate regulatory agencies. Confidentiality will be preserved to the fullest extent possible by the research team.

Data Sharing in Public Databases: There is a risk that the information in a scientific database can be traced back to a study participant, since even without any identifying information; genetic information is unique to each individual. We believe the chance of this happening is very small.

7. SAFETY & ADVERSE EVENTS

7.1 Definitions of Adverse Events

An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human research study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, clinical event, or disease, temporarily associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

AEs encompass clinical, physical and psychological harms. They occur most commonly in the context of biomedical research, although on occasion, they can occur in the context of social and behavioral research. AEs may be expected or unexpected.

AEs will be graded by a numerical score according to the defined NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) and version number specified in the protocol. AEs not specifically defined in the NCI CTCAE will be scored on the Adverse Event log according to the general guidelines provided by the NCI CTCAE and as outlined below.

- Grade 1: Mild
- Grade 2: Moderate

- Grade 3: Severe or medically significant but not immediately life threatening
- Grade 4: Life threatening consequences
- Grade 5: Death related to the adverse event

ICH Guideline E2A and the IRB define serious adverse events (SAE) as those events, occurring at any dose, which meets any of the following criteria:

- Results in death
- Immediately life-threatening
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Note: A “serious adverse event” is by definition an event that meets any of the above criteria. SAEs may or may not be related to the research project. A SAE determination does not require the event to be related to the research. That is, both events completely unrelated to the condition under study and events that are expected in the context of the condition under study may be serious adverse events, independent of relatedness to the study itself. As examples, a car accident requiring overnight hospitalization would be a SAE for any research participant; likewise, in a study investigating end-stage cancer care, any hospitalization or death would be a SAE, even if the event observed is a primary clinical endpoint of the study.

Underlying diseases should not be reported as AEs, but any increase in severity of the underlying disease which is not anticipated may be considered an AE. Elective procedures and/or elective hospitalizations (e.g., cosmetic surgery) should not be reported as an AE.

Patients should be instructed to advise physicians of suspected AEs after their enrollment in the study. AEs will be monitored from the time of study entry through 3 month follow-up. Events ongoing at the time of the 3 month follow-up will be documented as such. Information will be collected on all deaths and/or reintervention for 1 year post procedure.

7.2 Data Collection Procedures for Adverse Events

Each AE/SAE must be recorded in the subject's medical records and on a separate Adverse Event form in the CRF. Documentation must include a description of the event, including the

onset date, duration (start and end dates), severity, an assessment of cause, (Investigator's assessment of the relationship to the study treatment), any action taken, and the outcome.

Timely completion of CRFs is critical. All AEs should be recorded within 24 hours and SAEs within 24 hours of becoming aware of the event.

7.3 Reporting Procedures

For AEs, study personnel should complete the CRF AE within 72 hours of learning of the event. Additional event information (e.g. surgical records) should be entered into the CRF promptly upon receipt. For SAEs, study personnel must complete the CRF AE within 24 hours of learning of the event.

It is the responsibility of Principal Investigator or his designated representative to inform the IRB of SAEs as required by the University of Maryland IRB reporting requirements. All AEs and SAEs shall be reported to the Principal Investigator of this study.

7.4 Post-study Adverse Events

Information on post-study AEs will be collected on all deaths and/or reintervention for 3 month post procedure. The study will be halted for enrollment after one serious drug-related adverse event.

8. MONITORING PLAN

AE data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during a trial. Additionally, certain AEs must be reported in an expedited manner to allow for optimal monitoring of subject safety and care.

All subjects experiencing an AE, regardless of its relationship to study protocol, will be monitored until:

- The AE resolves or the symptoms or signs that constitute the AE return to baseline;
- Any abnormal laboratory values have returned to baseline;
- There is a satisfactory explanation other than the study protocol for the changes observed;
or
- Death.

DSMB oversight will be conducted in accordance with the Greenebaum Comprehensive Cancer Center standard operating procedures. It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. This study will be monitored by the UMGCCC Data and Safety Monitoring and Quality Assurance Committee and will follow the Data Safety and Monitoring Plan. Monitoring will be conducted as per the plan on file with the University of Maryland IRB.

9. STATISTICAL PLAN

The overall statistical design consists of two sequential phases, a pilot phase to establish procedures and parameters for performing the scans in humans, and a second phase design as a superiority study where we aim to test if the success rate is superior to 50%.

9.1 Primary and secondary endpoints.

The primary aim of this study is to establish a clinical workflow that is acceptable to patients and staff and results in approximately 75% usable scans. The primary endpoint will be a “successful” scan, defined as one where the signal-to-noise ratio (SNR) of the measured metabolite is at least 3 at one of the time points or a total SNR (as data summed over time points) of at least 36. We expect an approximately 75% success rate with this metric (informal communication with other centers, no published data are available).

Secondary endpoint: Any grade 3 or more adverse event.

In addition, we will estimate the population variability in the measured SNR; this estimate will be helpful for design of future larger trials. Operational endpoint, such as the total time of the procedure and the time from dissolution to injection, will be collated and reported as a median value with inter-quartile range.

The accuracy of the hyperpolarized metabolomic MRI to detect prostate cancer will be determined by comparing the positive or negative read on the MRI versus the presence or absence of cancer on the pathology. We will calculate the number of correctly called scans (ie positive scan in the setting of positive pathology, or negative scan in the setting of negative pathology) divided by total studies performed to generate an accuracy metric.

9.1 First phase – pilot study.

The pilot phase is exploratory rather than hypothesis driven. In this phase, we will enroll up to 10 patients and we will be optimizing settings of the infusion, scanner, endorectal coil, and clinical workflow. We will also be measuring performance with the key outcome of interest for quality assessment: SNR

9.2 Second phase – testing the achievable success rate.

Once the workflow and scanning protocol has been established, we will enter the second phase. For the single-stage second phase of the study, we use a Fleming-A'Hern [18, 19] design to test the null hypothesis (H_0) that the population success rate p_0 is 75% for the metabolic scan. The alternative hypothesis (H_1) is that the success rate is $p_1=50\%$. If we enroll 26 patients, we will have 83.7% power ($1-\beta$) to detect a difference (P_1-P_0) of - 0.2500 using a one-sided Fisher's exact test with a target significance level of $\alpha=0.0500$. The actual significance type I error rate achieved by this test is 0.0401. If the observed number of successful scans is less than or equal to 15, we will reject the null hypothesis. The sample size is estimated using binomial enumeration.

9.3 Second phase – stopping boundary for adverse events.

Sequential boundaries will be used to monitor the rate of any grade 3 or more adverse events [20]. The accrual will be halted if excessive numbers of high-grade toxicities are seen, that is, if the number of these toxicities is equal to or exceeds b_n out of n patients with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 5% when the rate of any grade 3 or more adverse events is equal to the acceptable rate, also chosen at 5%. If the true rate of grade 3 or more adverse events is 30% or higher, the probability of early stopping is 96%. Statistically, under these assumptions, this will happen on average after enrolling 9 patients in the second phase of the study.

This study will be halted for enrollment if there is one serious drug-related adverse event.

If the study is suspended or terminated for safety reasons, the PI will inform the regulatory authorities of the suspension or termination of the study and the reasons for the action. The PI is responsible for promptly informing the IRB/EC and providing the reasons for the suspension or termination of the study.

No. of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Boundary, b_n	-	2	2	2	2	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	5	5	5	5

9.4 Data Analysis.

The primary endpoint will be the proportion of “successful” scans as defined above. This proportion will be reported with exact 90% confidence limits. Other endpoints will be

summarized using descriptive statistics. We will report all metrics on an “intention-to-scan” principle with all consented patients included in the study population, as well as in the population who actually had the injection of Pyruvate. Patients who irrespective of the reason are not scanned will be counted as “failed” scans in the ITT analysis.

All adverse events irrespective of grade will be recorded and reported with an attribution to the scan procedure. A statistical analysis plan (SAP) will be developed for each analysis before commencement.

10. DATA HANDLING & RECORD KEEPING

10.1 Confidentiality & Security

All research study participants will be assigned a unique ID number. This number will be generated by a computerized random number generator. Study documentation and data will be labeled with participants’ unique ID numbers to ensure confidentiality. Only the Principal Investigator and study team members will have access to the key.

All paper documents related to the research study will be stored in a locked file cabinet in a locked room at the University of Maryland. Electronic study data and documents will be stored on encrypted password protected computers located in a locked room at the University of Maryland Division of Urology at the University of Maryland Medical Center.

10.2 Training

The Principal Investigator will conduct training before initiating the study to develop a common understanding of the clinical protocol, CRFs, and study procedures among research team members.

10.3 Case Report Forms & Source Documents

Case Report Forms (CRFs) will be used to collect all patient data during the study. CRFs for a patient should be completed within 7 days of evaluations. The Principal Investigator and/or co-investigator shall confirm data is accurate by reviewing with the provider collecting the data.

11. REFERENCES

1. Siddiqui, M.M., Rais-Bahrami, S., Truong, H., Stamatakis, L., Vourganti, S., Nix, J., Hoang, A.N., Walton-Diaz, A., Shuch, B., Weintraub, M., Kruecker, J., Amalou, H., Turkbey, B., Merino, M.J., Choyke, P.L., Wood, B.J., and Pinto, P.A., *Magnetic resonance imaging/ultrasound-fusion biopsy significantly upgrades prostate cancer versus systematic 12-core transrectal ultrasound biopsy*. Eur Urol, 2013. **64**(5): p. 713-9.
2. Siddiqui, M.M., Rais-Bahrami, S., Turkbey, B., George, A.K., Rothwax, J., Shakir, N., Okoro, C., Raskolnikov, D., Parnes, H.L., Linehan, W.M., Merino, M.J., Simon, R.M., Choyke, P.L., Wood, B.J., and Pinto, P.A., *Comparison of MR/ultrasound fusion-guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate cancer*. JAMA, 2015. **313**(4): p. 390-7.
3. Nelson, S.J., Kurhanewicz, J., Vigneron, D.B., Larson, P.E., Harzstark, A.L., Ferrone, M., van Criekinge, M., Chang, J.W., Bok, R., Park, I., Reed, G., Carvajal, L., Small, E.J., Munster, P., Weinberg, V.K., Ardenkjaer-Larsen, J.H., Chen, A.P., Hurd, R.E., Odegardstuen, L.I., Robb, F.J., Tropp, J., and Murray, J.A., *Metabolic imaging of patients with prostate cancer using hyperpolarized [1-(1)(3)C]pyruvate*. Sci Transl Med, 2013. **5**(198): p. 198ra108.
4. Franklin, R.B., Ma, J., Zou, J., Guan, Z., Kukoyi, B.I., Feng, P., and Costello, L.C., *Human ZIP1 is a major zinc uptake transporter for the accumulation of zinc in prostate cells*. J Inorg Biochem, 2003. **96**(2-3): p. 435-42.
5. Sciarra, A., Panebianco, V., Ciccariello, M., Salciccia, S., Lisi, D., Osimani, M., Alfarone, A., Gentilucci, A., Parente, U., Passariello, R., and Gentile, V., *Magnetic resonance spectroscopic imaging (1H-MRSI) and dynamic contrast-enhanced magnetic resonance (DCE-MRI): pattern changes from inflammation to prostate cancer*. Cancer investigation, 2010. **28**(4): p. 424-432.
6. Priolo, C., Pyne, S., Rose, J., Regan, E.R., Zadra, G., Photopoulos, C., Cacciatore, S., Schultz, D., Scaglia, N., McDunn, J., De Marzo, A.M., and Loda, M., *AKT1 and MYC Induce Distinctive Metabolic Fingerprints in Human Prostate Cancer*. Cancer research, 2014. **74**(24): p. 7198-7204.
7. Wu, X., Daniels, G., Lee, P., and Monaco, M.E., *Lipid metabolism in prostate cancer*. American journal of clinical and experimental urology, 2014. **2**(2): p. 111-120.
8. Shiraishi, T., Verdone, J.E., Huang, J., Kahlert, U.D., Hernandez, J.R., Torga, G., Zarif, J.C., Epstein, T., Gatenby, R., McCartney, A., Elisseeff, J.H., Mooney, S.M., An, S.S., and Pienta, K.J., *Glycolysis is the primary bioenergetic pathway for cell motility and*

- cytoskeletal remodeling in human prostate and breast cancer cells*. Oncotarget, 2015. **6**(1): p. 130-143.
9. Wetter, A., Engl, T.A., Nadjmabadi, D., Fliessbach, K., Lehnert, T., Gurung, J., Beecken, W.-D., and Vogl, T.J., *Combined MRI and MR spectroscopy of the prostate before radical prostatectomy*. American Journal of Roentgenology, 2006. **187**(3): p. 724-730.
 10. Reinsberg, S.A., Payne, G.S., Riches, S.F., Ashley, S., Brewster, J.M., Morgan, V.A., and deSouza, N.M., *Combined use of diffusion-weighted MRI and ¹H MR spectroscopy to increase accuracy in prostate cancer detection*. American Journal of Roentgenology, 2007. **188**(1): p. 91-98.
 11. Singh, A.K., Krieger, A., Lattouf, J.-B., Guion, P., Grubb, R.L., Albert, P.S., Metzger, G., Ullman, K., Smith, S., Fichtinger, G., Ocak, I., Choyke, P., Ménard, C., and Coleman, J., *Patient selection determines the prostate cancer yield of dynamic contrast-enhanced magnetic resonance imaging-guided transrectal biopsies in a closed 3-Tesla scanner*. BJU international, 2008. **101**(2): p. 181-185.
 12. Ardenkjær-Larsen, J.H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M.H., Servin, R., Thaning, M., and Golman, K., *Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR*. PNAS USA, 2003. **100**(18): p. 10158-10163. PMC193532
 13. Kurhanewicz, J., Vigneron, D.B., Brindle, K., Chekmenev, E.Y., Comment, A., Cunningham, C.H., Deberardinis, R.J., Green, G.G., Leach, M.O., Rajan, S.S., Rizi, R.R., Ross, B.D., Warren, W.S., and Malloy, C.R., *Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research*. Neoplasia, 2011. **13**(2): p. 81-97. PMC3033588
 14. Albers, M.J., Bok, R., Chen, A.P., Cunningham, C.H., Zierhut, M.L., Zhang, V.Y., Kohler, S.J., Tropp, J., Hurd, R.E., Yen, Y.-F., Nelson, S.J., Vigneron, D.B., and Kurhanewicz, J., *Hyperpolarized ¹³C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading*. Cancer research, 2008. **68**(20): p. 8607-8615.
 15. Day, S.E., Kettunen, M.I., Gallagher, F.A., Hu, D.-E., Lerche, M., Wolber, J., Golman, K., Ardenkjaer-Larsen, J.H., and Brindle, K.M., *Detecting tumor response to treatment using hyperpolarized ¹³C magnetic resonance imaging and spectroscopy*. Nature medicine, 2007. **13**(11): p. 1382-7.
 16. Nelson, S.J., Kurhanewicz, J., Vigneron, D.B., Larson, P.E.Z., Harzstark, A.L., Ferrone, M., Van Criekinge, M., Chang, J.W., Bok, R., Park, I., Reed, G., Carvajal, L., Small, E.J., Munster, P., Weinberg, V.K., Ardenkjaer-Larsen, J.H., Chen, A.P., Hurd, R.E., Odegardstuen, L.-I., Robb, F.J., Tropp, J., and Murray, J.A., *Metabolic Imaging of*

- Patients with Prostate Cancer Using Hyperpolarized [1-13C]Pyruvate*. Science Translational Medicine, 2013. **5**(198): p. 198ra108.
17. Larson PEZ, Chen HY, Gordon JW, Korn N, Maidens J, Arcak M, Tang S, Crieckinge M, Carvajal L, Mammoli D, Bok R, Aggarwal R, Ferrone M, Slater JB, Nelson SJ, Kurhanewicz J, Vigneron DB. Investigation of analysis methods for hyperpolarized 13C-pyruvate metabolic MRI in prostate cancer patients. NMR in biomedicine. 2018;31(11):e3997. doi: 10.1002/nbm.3997. PubMed PMID: 30230646; PMCID: PMC6392436.
 18. A'Hern, R. P. A. (2001). Sample size tables for exact single-stage phase II designs. Statistics in Medicine 20: 859-866.
 19. Fleming, T. R. (1982). One-sample multiple testing procedure for Phase II clinical trials. Biometrics, 38: 143-151.
 20. Ivanova, A., Qaqish, B.F., and Schell, M.J. (2005). Continuous toxicity monitoring in phase II trials in oncology. Biometrics 61: 540-545.

12. APPENDIX

13C Clamshell_Endo IEC 60601_2_33_2010 Test Report
(Endo_and_ClamCoil_IEC_Report.pdf)