Amendment

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Protocol Title: Pilot Study of Eovist® (Gadoxetate) Enhanced MRI for the Detection of Prostate Cancer

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* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: NA

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Drug: Eovist® (**Gadoxetate Disodium**)
Manufacturer: Bayer Healthcare Pharmaceuticals Inc

PRECIS

Background:

- Prostate cancer is the most common non-cutaneous malignancy among men in the western world. Prognostic biomarkers would be useful in stratifying patients to different treatments.
- The expression of a testosterone membrane transporter, OATP1B3, is associated with shorter time to progression after hormonal ablation therapy and shorter overall survival in prostate cancer patients. 52% of localized prostate cancer lesions express OATP1B3, while 92% of prostate cancer metastases requiring hormonal ablation treatment, express OATP1B3 in soft tissue lesions. Expression of OATP1B3 also correlates with Gleason grade.
- Current imaging methods cannot predict treatment failure or resistance.
- Gadoxetate disodium (Gd-EOB-DTPA) (Eovist®, Bayer HealthCare Pharmaceuticals Inc. Pittsburgh, PA) is an MR imaging agent which is FDA-approved gadolinium chelate for detecting hepatocellular carcinoma (HCC), as normal hepatocytes express OATP1B3 while most hepatocellular carcinomas do not. However, those HCC's that do take up Eovist® have been shown to express OATP1B3.
- Eovist® may be useful to evaluate OATP1B3 status in patients with prostate cancer and may therefore serve as a prognostic and treatment biomarker.

Primary Objective:

- Evaluate the uptake and retention of Eovist® in prostate cancers.

Eligibility:

- Male subjects ≥18 years old
- ECOG Performance score of 0 to 2
- Subjects with clinically localized prostate cancer must have image guided biopsy confirmed prostate cancer and sufficient tissue available for OATP1B3 IHC.
- Subjects with advanced disease who have failed hormone therapy and who have sufficient tissue from a soft tissue or metastatic bone lesion (measuring ≥1.5cm in diameter at CT or MRI scan) available for OATP1B3 IHC.

  or

- Subjects, for whom tissue is not available, must have a soft tissue or metastatic bone lesion that can be biopsied and be willing to undergo percutaneous biopsy to obtain tissue for OATP1B3 expression.

Design:

- This pilot study will accrue 25 subjects divided into two arms: 10 evaluable subjects with localized prostate cancer and 15 evaluable subjects with advanced disease
- Each subject will receive a single IV dose of Eovist® by bolus injection
- All subjects will undergo MRI prior to and immediately after, 10, 20 and 60 minutes post-Eovist® injection

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1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary Objective

- Evaluate the uptake and retention of Eovist® in prostate cancers

1.1.2 Secondary Objective

- Evaluate MR contrast enhancement parameters following Eovist® injection with respect to Gleason Score and serum PSA levels.

1.1.3 Exploratory Objective

- Evaluate MR contrast enhancement parameters with respect to OATP1B3 expression.

1.2 Background and Rationale

1.2.1 Study Disease: Prostate Cancer

Prostate cancer is the second most common cause of cancer death in men in the United States. The American Cancer Society estimates that there were 217,730 new cases and 32,050 deaths due to prostate cancer (CaP) in the United States in 2010 [1]. Despite screening with PSA, many patients are diagnosed with advanced stage disease. Androgen deprivation therapy (ADT) is the frontline treatment approach for advanced prostate cancer and may be accomplished by medical or surgical castration. Although the majority of prostate cancer patients initially respond to ADT, disease almost invariably progresses eventually to castration-resistant prostate cancer.

1.2.2 Castration Resistant Prostate Cancer (CRPC)

Normal and cancerous growth of the prostate depends on androgen receptor (AR) expression and function. Activation and translocation of the AR by androgens leads to transcriptional activity of AR target genes to induce cell growth and maintain homeostasis. In addition, signaling of growth factors or transport of hormones may have an important role in carcinogenesis and in progression to castrate-resistant disease. Understanding the molecular events that lead to castration resistance is essential in developing successful treatments. Multiple mechanisms involved in the development of CRPC have been proposed, including AR over-expression, AR gene amplification, alteration in AR structure or function, intratumoral androgenic steroid synthesis, ligand-independent AR reactivation by growth factor pathways, as well as AR-independent mechanisms [2]. While many studies have revolved around the AR and its involvement in the androgen signaling axis, relatively little is known about the role of testosterone transport and androgen transporters such as OATP1B3 in prostate cancer. Emerging data suggest a potential role for these steroid transporters in mediating the uptake of androgen into CaP cells and thereby influencing the clinical response to androgen suppression.

1.2.3 OATP1B3 Transports Testosterone and affects clinical outcomes in CaP

Previous studies have demonstrated that single nucleotide polymorphisms (SNPs) in *SLCO* genes, which encode OATPs, can markedly alter testosterone transport efficiency. Recently, the Figg lab has specifically evaluated these SNPs and their association with androgen

transport. They showed that a common SLCO1B3 GG/AA haplotype is associated with impaired testosterone transport and improved survival in patients with prostatic cancer [3]. The study examined (a) the *SLCO1B3* genotype in cancer cells as well as the uptake of testosterone by cells transfected with genetic variants of SLCO1B3; (b) the expression of OATP1B3 in normal prostate, benign prostatic hyperplasia, and prostatic cancer; and (c) the role of SLCO1B3 haplotype on the clinical outcome of Caucasian patients with androgen-independent prostatic cancer. Cells transfected with wild-type (334T/699G) SLCO1B3, actively transported testosterone, whereas its uptake was impaired in cells transfected with a gene carrying both 334G and 699A single nucleotide polymorphisms. Approximately half of localized prostatic cancers over-express OATP1B3 compared with normal or benign hyperplastic tissue; patients with SLCO1B3 334GG/699AA haplotype showed longer median survival (8.5 versus 6.4 years; $P = 0.020$) and improved survival probability at 10 years (42% versus 23%; $P < 0.023$) than patients carrying TT/AA and TG/GA haplotypes. The common SLCO1B3 GG/AA haplotype is associated with impaired testosterone transport and improved survival in patients with prostatic cancer [3].

In a follow-up study, they further showed that the polymorphism that increases testosterone transport was associated with a shorter time to androgen independence in patients with prostate cancer who are treated with ADT. The study examined the association between this SLCO1B3 polymorphism and time from ADT to androgen independence, ADT to prostate-specific antigen (PSA) nadir and PSA nadir to androgen independence in 68 Caucasian patients with advanced prostate cancer with metastatic disease (D2) or biochemical failure without metastases (D0) who were treated with ADT. When examined separately, patients with the 334T allele tended to have a shorter time to androgen independence in the D0 ($P = 0.11$) and D2 ($P = 0.18$) groups. Combining these groups and stratifying by stage yielded a statistically significant shorter time to androgen independence in those patients carrying the T allele ($P = 0.048$) [4].

Finally, another study by Wright et al found that CRPC metastases demonstrate increased expression of SLCO genes vs. primary CaP. SLCO1B3 was highly expressed in CRPC metastases vs. untreated CaP (3.6 fold, p=0.0517) and SLCO1B3 SNP rs4149117 (HR 1.76, 95% CI 1.00 - 3.08) had an increased risk of CaP-specific mortality. In their study, CRPC metastases showed increased expression of SLCO genes in comparison with primary prostate cancer. Genetic variants of SLCO1B3 and SLCO2B1 found to be associated with CaP-specific mortality. Expression and genetic variation of SLCO genes which alter androgen uptake was proposed to be important in prostate cancer outcomes and this may allow stratification of patients to more aggressive hormonal therapy or earlier incorporation of non-hormonal based treatment strategies [5].

1.2.4 Rationale

No currently used imaging method evaluates the status of the OATP1B3 transporter. Such a method might be important in stratifying risk factors in patients, both with localized and with metastatic disease.

Eovist® (gadoxetate disodium) is a gadolinium-based contrast agent which was approved by the FDA in 2008 [6]. It is indicated for intravenous use in T1-weighted magnetic resonance imaging (MRI) of the liver to detect and characterize lesions in adults with known or suspected focal liver disease, particularly in the diagnosis of hepatocellular carcinoma (HCC). The agent

is taken up by normal hepatocytes and lesions that contain normal hepatocytes (e.g. focal nodular hyperplasia). The majority of HCCs do not take up Eovist® and so the agent has become useful in the workup of suspected HCC. However, some HCC tumors do take up Eovist® and this uptake has been correlated with their OATP1B3 expression [7, 8].

The agent is injected intravenously. Because it is a low molecular weight imaging agent it rapidly enhances all vascular organs and structures. However, it was not retained by non OATP1B3 expressing tissue and so at 20 minutes a correlation was made between OATP1B3 expressing lesions (persistent enhancement) and non OATP1B3 expressing lesions (washout of enhancement).

Thus, Eovist® will be used in patients with localized and metastatic prostate cancer in order to determine if this method is feasible to observe differences in uptake times in prostate cancers and whether these differences correlate with OATP1B3 expression in prostate cancer and therefore, might serve as a predictive biomarker.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

2.1.1.1 Subject is ≥18 years old.

2.1.1.2 Subjects with clinically localized prostate cancer (outside pathology is acceptable) must have image guided biopsy confirmed prostate cancer and sufficient tissue available (obtained before or after 20 weeks of Eovist® injection) for OATP1B3 expression.

2.1.1.3 Subjects with advanced disease who have failed hormone therapy and who have sufficient tissue (obtained before or after 20 weeks of Eovist® injection) from a soft tissue or metastatic bone lesion (measuring ≥1.5cm in diameter at CT or MRI scan) available for OATP1B3 expression.

or

2.1.1.4 Subjects, for whom tissue is not available, must have a soft tissue or metastatic bone lesion that can be biopsied and be willing to undergo percutaneous biopsy to obtain tissue for OATP1B3 expression.

2.1.1.5 ECOG performance status ≤2

2.1.1.6 Serum creatinine within 3 weeks prior to Eovist MRI ≤1.8mg/dl and estimated glomerular filtration rate (eGFR) must be >30 ml/min/1.73m^2.

2.1.1.7 Patients must have normal liver function as defined below:

- total bilirubin< 2X normal institutional limits or >3.0 mg/dl in patients with Gilbert's syndrome
- AST(SGOT) and ALT(SGPT)≤3 X institutional upper limit of normal

2.1.1.8 Ability of subject to sign a written informed consent document

2.1.2 Exclusion Criteria

2.1.2.1 Subjects with known hypersensitivity and allergy to gadolinium contrast agents

2.1.2.2 Subjects with any coexisting medical or psychiatric condition that is likely to interfere with study procedures and/or results

2.1.2.3 Subjects with severe claustrophobia unresponsive to oral anxiolytics

2.1.2.4 Subjects with contraindications to MRI

2.1.2.5 Subjects weighing >136 kg (weight limit for scanner table)

2.1.2.6 Subjects with pacemakers, cerebral aneurysm clips, shrapnel injury, or other implanted electronic devices or metal not compatible with MRI

2.1.2.7 Subjects with other medical conditions deemed by the principle investigator (or associates) to make the subject ineligible for protocol procedures

2.1.2.8 Subjects who will have a delay in clinically indicated radiation therapy due to the interval between Eovist® MRI imaging and biopsy

2.2 Screening Evaluation

Subjects will be seen in either the UOB, ROB or MOB clinics by members of Dr. Peter Pinto`s, Dr. Aradhana Kaushal`s and/or Dr. William Dahut`s teams, respectively. Conventional imaging studies confirming presence of metastatic soft tissue or metastatic bone prostate cancer lesions (outside imaging studies are acceptable for study entry) will be reviewed by the Molecular Imaging Program staff.

2.2.1 Screening tests:

Screening Test must be completed within 21 days prior to the injection of Eovist®

2.2.1.1 Evaluations:

- vital signs (BP, HR, RR, & Temperature)
- clinical laboratory assessments (CBC w/diff, Acute Care Panel, Hepatic Panel, Mineral Panel, & PSA)
- OATP1B3 genotype analysis of blood samples will be done in Dr. William D. Figg`s lab; results will not be a part of the study eligibility criteria.

2.3 Registration Procedures

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 Study Design

This is an open-label, single-center pilot study. Twenty evaluable subjects will be enrolled, 10 subjects with localized prostate cancer and 10 subjects with advanced prostate cancer with soft tissue or bone involvement. A subject is considered evaluable if he completes pre and post Eovist® injection MRI scans and has sufficient tissue for OATP1B3 IHC.

- Localized disease:
    - If existing pathologic tissue can be linked with confidence to an MRI-defined lesion (i.e. pathologic specimen is labeled as to location) then that tissue can be used for OATP1B3 expression.
    - Otherwise the tissue obtained during image guided biopsy at NCI will be used for OATP1B3 expression testing.
    - Prostatectomy specimens may be a source of tissue if the above sources are not available and the patient undergoes prostatectomy.
- Advanced disease:
    - If clinically acquired tissue is not available, the patient must be willing to have a percutaneous needle biopsy of a soft tissue lesion or metastatic bone lesion (measuring ≥1.5cm in diameter at CT or MRI scan).

All tissue samples (either biopsy specimens from localized intraprostatic lesions or prostatectomy specimens in the localized disease arm or biopsy specimens from the soft tissue or metastatic bone lesions in the advanced disease arm) for immunohistochemical (IHC) analysis must be acquired within ±20 weeks (assuming no intervening therapy) of Eovist® imaging.

3.1.1 Eovist® enhanced MRI

MRI will be performed in the Molecular Imaging Clinic. Pre contrast T1, T2 weighted scans with or without endorectal coil will be obtained through the prostate gland, bone metastasis or soft tissue metastasis (usually a lymph node) selected as the target lesion. Then 0.1 ml/kg Eovist® will be administered IV. Scans will be obtained immediately, 10, 20 and 60 minutes after injection using the same settings. Contrast enhancement ratios will be determined at each time point (Section 3.3).

3.1.2 OATP1B3 Genotyping

Genotyping experiments will be performed using genomic DNA isolated from stored frozen serum using the QIAmp DNA blood mini-kit (Qiagen, Inc, Valencia, CA). This method has been previously utilized for the isolation of genomic DNA in the Figg laboratory [3, 4] and has resulted in DNA yields of approximately 50-400 ng/0.5ml of serum.

Genomic DNA will be amplified using *Taq* polymerase. Depending on the gene, single nucleotide polymorphisms in the CYP450 alleles will be identified using direct sequencing. Dr. Figg's laboratory has previously published methods for genotyping the OATP1B3 alleles under study [3, 4].

All biosamples have been given a specimen code number on arrival in the laboratory. All further manipulation of the data is performed using the code number. Only named investigators will have access to this information, but most information will be stored and analyzed by the statistics section of the CCR.

3.1.3 Pathology

3.1.3.1 Surgical Pathology

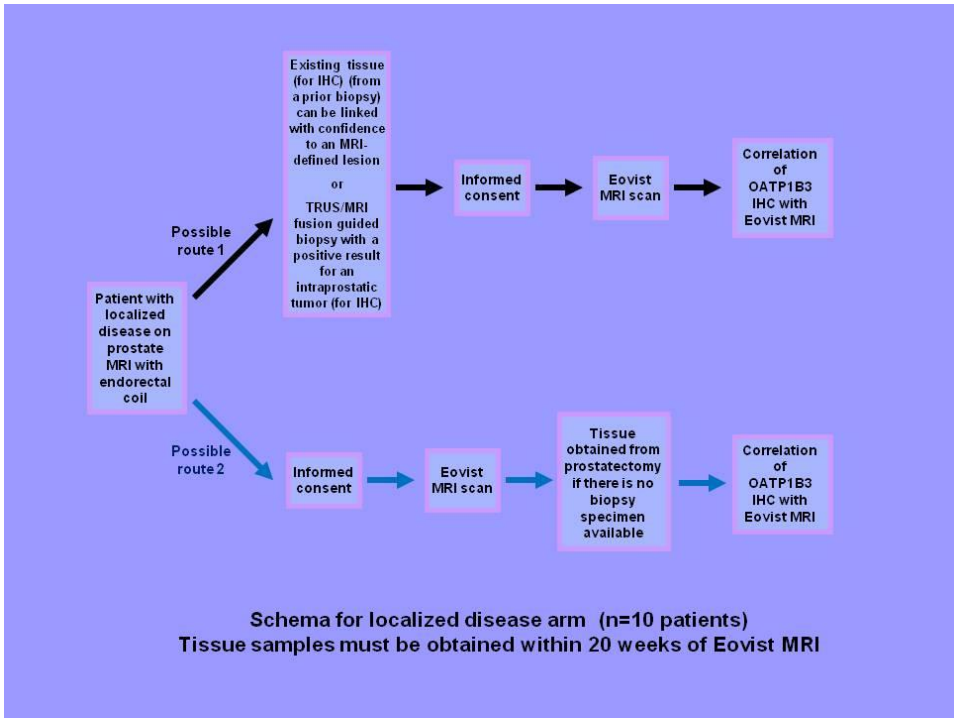
For the localized disease arm, biopsy specimens obtained from intraprostatic lesions or prostatectomy specimens will undergo standard preparation and sectioning in the Laboratory of Pathology for pathological diagnosis. The biopsy procedures for the localized disease arm will be part of standard of care.

For the advanced disease arm, biopsy specimens obtained from distant lesions will undergo standard preparation and sectioning in the Laboratory of Pathology for pathological diagnosis. The biopsy procedure for the advanced disease arm will be research biopsies and will be performed under imaging (ultrasound or CT) guidance.

3.1.4 Immunohistochemistry

Immunohistochemistry analysis will include exploratory assessment of OATP1B3 expression. Analyses will be performed on tumor from all specimens obtained and, will be performed at NIH. The Figg Lab has previously published immunofluorescence methods to detect OATP1B3 in prostate tissue sections isolated from men with benign prostatic hyperplasia, and prostate cancer [3]. The assay was able to distinguish between expressing and non-expressing tumors using a primary OATP1B3 antibody purchased from Santa Cruz Biotech. This will be used for IHC analysis. Moreover, we are currently developing better methods of detection in collaboration with Dr. Scott Lucia (University of Colorado Denver). We are also collaborating with Dr. Wooin Lee (University of Kentucky) who has recently published IHC methods using antibodies developed in-house. This is a topic which is still under investigation. The scope of collaboration with these groups will be limited to data exchange, but will not include sending out tissue samples.

3.2 Study Schema



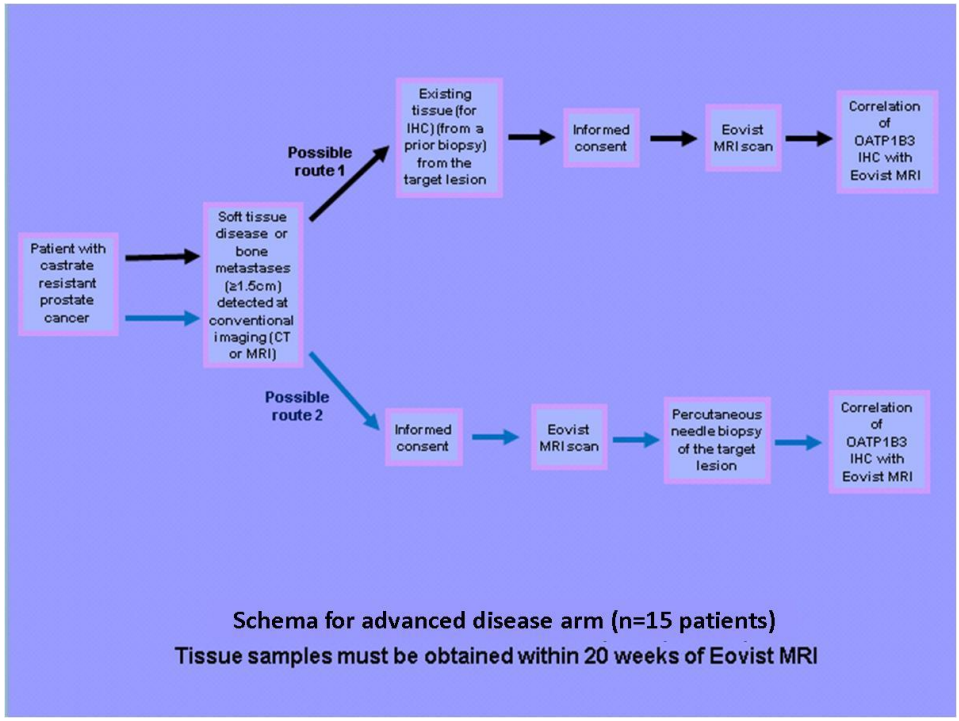


Figure 1: Study Schemas for localized and advanced disease arms.

Note: Tissue samples must be obtained within ±20 weeks of Eovist® MRI

3.3 Study Drug Administration/Administration of Eovist®

Under the supervision of PI or designated personnel, each subject will receive a single IV dose of Eovist® by bolus injection at a rate of approximately 2 ml/second at a dose of 0.1ml/kg. The injection will be followed by a 10-ml saline flush (sodium chloride IV infusion 0.9% w/v) over about10seconds.

The injection site will be evaluated before and after injection, to assess for the presence of signs of local irritation.

A member of the MIP clinical team will be in attendance during the injection. In the event of an emergency, such as an allergic reaction, immediate treatment will be initiated using emergency medication available in the Molecular Imaging Clinic. If the subject requires admission due to the severity of the reaction, the subject will be admitted to UOB, MOB or CCMD services for observation as the clinical situation dictates.

3.4 Dose Modifications

If an adverse reaction is noted during the infusion, the infusion will be discontinued

3.5 Correlative Studies

3.5.1 Imaging Correlation

A volume of interest (VOI) will be drawn over the target lesion in the baseline and in post-Eovist® injection MR images and the amount of contrast enhancement will be recorded by using dedicated image analysis software (MIM 5.4 Software, MIM Software Inc. Cleveland, OH, USA*)*. Enhancement ratios [(signal enhancement after enhancement-signal enhancement before enhancement)/signal intensity before enhancement] will be calculated on pre and post-injection T1W MR images.

3.5.2 Pathologic and Imaging Correlation

Standard of care biopsy is planned for subjects enrolled in the localized disease arm of this study. For patients enrolled in the advanced disease arm, the biopsies will be obtained for research purposes.

In case the new imaging agent shows an unexpected finding, this will be correlated with conventional imaging. The physicians of the patient will use best clinical judgment in determining whether a finding will influence the surgical procedure.

3.5.3 Histological Correlations

OATP1B3 expression will be confirmed by staining for OATP1B3 in tissue samples on an investigational basis. IHC analysis will be conducted by one NCI pathologist and will be based on grade of positivity (0 (no staining), 1 (weak staining), 2 (moderate staining) or 3 (strong staining)).

3.5.4 Safety Assessments

Safety assessments will obtained during the course of the study are summarized in the Study Calendar (Section **3.6**).

3.6 Study Calendar

The timing of study events in the following descriptions is relative to the administration of EOVIST®.

Procedure	Screening[a]	Baseline[b]	MR imaging			Scheduled Post-MRI[c]
			Just prior to MRI	Pre-injection MRI scan	Post injection MRI scan	
Informed consent	X					
Study entry criteria	X					
Demographic information	X					
Medical history and concurrent diseases	X					
Prior/concomitant medications	X					
Injection site monitoring				X	X[d]	X
Vital signs	X	X			X[e]	
Acute Care Panel, Hepatic Panel, Mineral Panel, PSA, & CBC	X[f]					
OATP1B3 Genotype analysis	X[g]					
Biopsy specimen available for OATP1B3 expression	X[h, i]					
Intravenous line started			X			
Eovist® injection				X		
Adverse Event Monitoring			X	X	X	X
MR Imaging				X	X[j]	
Biopsy Procedure						X[k, l]
Prostatectomy						X[m]
OATP expression and Comparative Analyses						X

a: Within 21 days of administrations of Eovist®

b: The day of MR imaging until just prior to administration of Eovist ®

c. Timing of events is relative to administration of Eovist®

d: 5 minutes post-Eovist® injection

e: At the end of all scanning

f: Clinical blood sample assessments must be performed within 21 days prior to administration of Eovist®.

g: Will be performed at Dr. William D. Figg`s Lab.

h: For the localized disease arm, existing pathologic tissue (obtained <20 weeks before Eovist MRI) will be screened to see if it can be linked to MRI visible lesion in the patient candidate for Eovist MRI. Otherwise the tissue obtained from image guided biopsy (standard of care) at NCI will be used for OATP1B3 expression.

i: For the advanced disease arm, availability of the previously (<20 weeks) obtained clinically acquired tissue will be checked.

j: Immediately, 10, 20, & 60 minutes post-Eovist® injection

k: To be conducted within <20 weeks after Eovist® enhanced MRI

l: This applies for the advanced disease arm and will be conducted from a soft tissue or metastatic bone lesion (measuring ≥1.5cm in diameter).

m: This applies for the localized disease arm and will serve as a source of tissue only if previously acquired biopsy (confidently linked to an MRI visible lesion) or image guided standard of care biopsy at NCI will not be available.

3.6.1 Study Periods

3.6.1.1 Screening Period

A screening visit will be performed within 21 days before administration of Eovist®. Subjects will be permitted to continue taking any routine or necessary medication.

All subjects must satisfy all the eligibility criteria listed in Section **2.1**. Signed and dated informed consent must be obtained from all subjects before any study-specific procedures are performed.

The following data will be collected:

- Date of birth
- Weight
- Height
- Prior and concurrent medications
- Medical history and concurrent diseases
- Results of screening tests: laboratory tests

Personal data (including contact information) will be collected with the subject's permission and only to the extent that is necessary for the purposes of the study. Blood samples for laboratory tests will be drawn. Vital signs (i.e., systolic and diastolic blood pressure, heart rate, body temperature, and respiration rate) will be recorded.

3.6.1.2 Baseline Period

The baseline period is defined as the day of MR imaging until just prior to administration of Eovist®. At baseline, concomitant medication and pre-administration events will be recorded.

A cannula (or indwelling catheter) will be placed preferably into an antecubital vein for blood sampling and imaging agent administration. Blood samples for serum biochemistry and hematology will be drawn. Vital signs (i.e., systolic and diastolic blood pressure, heart rate, body temperature, and respiration rate) will be recorded.

If the screening clinical blood sample assessments are performed within 48 hours of administration of Eovist®, they do not need to be repeated.

3.6.1.3 MR Imaging Period

Prior to entering the MRI scanner the patient will answer the standard MRI safety checklist administered to all patients undergoing MRI in the Clinical Center to insure that it is safe to perform an MRI. The patient will be placed into the magnet in supine position and an intravenous line will be placed. A 32 channel cardiac coil will be used for MR imaging. An endorectal coil will be used for localized disease arm.

The following scans will be obtained:

- Scout view of the pelvis to check positioning of the cardiac coil.
- T1 weighted MRI of abdomen and pelvis.
- T2 weighted MRI of abdomen and pelvis.
- Total nominal time: 40 minutes +/- 5 minutes for setup and clean-up +/- 10 minutes in case any sequence needs to be repeated due to unforeseen events such as patient motion during imaging acquisition. At any given time the patient will be required to lie still for 5-7 minutes.

After completion of the pre-injection MRI scan, patient will have the Eovist® injection as detailed above in section **3.3**. The patient will be assessed for adverse events 15 minutes post-Eovist® Injection and if they are stable, the same MR imaging protocol (except for the scout view) specified above will be performed immediately after injection and at approximately 10^{th}, 20^{th} and 60^{th} minute time points after injection with the same MRI set up.

Following imaging acquisition, the data will be transferred to the clinical PACS system for storage.

3.6.1.4 Post-Eovist® Enhanced MR Imaging Period

A final set of vital signs and AE assessment will be performed at the end of the 60 minute MRI scan.

Within 20 weeks of the Eovist® enhanced MRI the biopsy procedure will be performed.

3.6.1.5 Clinical Laboratory Evaluations

The clinical laboratory variables assessed in this study are displayed in Table 1; they will be measured at the NIH Clinical Center laboratory.

Table 1 Clinical Laboratory Variables

Serum Biochemistry	Hematology

Alanine aminotransferase	Hematocrit
Albumin	Hemoglobin
Alkaline phosphatase	Platelet count
Aspartate aminotransferase	Red blood cell count
Bilirubin (total, direct)	White blood cell (WBC) count
Calcium	
Chloride	
Creatinine	
Glucose	
Potassium	
Sodium	
Urea nitrogen	
PSA	

Blood samples will be obtained for assessment of serum biochemistry and hematology parameters as described in the Study Calendar (Section **3.6**). The maximum amount of blood taken for these tests will not be more than 60 ml.

Interpretation and follow-up of abnormal laboratory test results should be conducted in conjunction with the clinical situation of the subject and in consultation with the referring physician.

3.6.1.6 Vital Signs

Vital signs will be measured at various pre- and post-injection time points described in the Study Calendar. Vital sign parameters include measurements of systolic and diastolic BP, heart rate, temperature and respiratory rate.

The interpretation and follow-up of abnormal vital signs results should be conducted on a case by case basis in conjunction with the individual clinical situation.

3.6.1.7 Injection Site Monitoring

Injection site monitoring will be performed prior to administration of the imaging agent and at the 5th minute post-injection described in the Study Calendar (Section **3.6**). Any abnormal findings during this period will be recorded as AEs on the research record. Abnormal injection site findings include, but are not limited to, pharmaceutical extravasation, bleeding, hematoma, redness and infection.

Any abnormal finding that is new or represents a worsening from baseline is an AE. Once AE notification is decided upon, investigators are required to follow the procedure described for AE notification and document the abnormal finding in the subject's research record.

3.6.1.8 Pre-administration events

The presence of any pre-administration event (baseline signs and symptoms present just before Eovist® administration) will be recorded in C3D

The following information will also be recorded:

- The date and time of evaluation
- The onset time
- The resolution time or duration
- Action taken
- Status of symptom
- Intensity

3.7 Surgical Guidelines

As part of their standard of care for their disease, subjects will have biopsy within 20 weeks of Eovist® enhanced MRI. Pathologic specimens will be obtained for histological assessment. Further details are provided in Section **3.1.4**.

In the event that the Eovist® enhanced MRI scan demonstrates an unexpected finding, it will be correlated with conventional imaging. While Eovist® enhanced MRI findings will not be used to direct therapy, the referring physician(s) will be made aware of any such findings, and may perform additional tests to clarify findings.

3.8 Radiation Therapy Guidelines

The patient should not undergo radiation therapy to the target lesion between biopsy and Eovist® MRI imaging. However, clinically indicated radiation therapy cannot be delayed by the biopsy after Eovist® enhanced MRI.

3.9 Off-Study Criteria

Patients will be taken off study once all study procedures are complete. In cases where a subject withdraws or is withdrawn from the study after administration of Eovist® and before biopsy is performed, a replacement subject will be enrolled.

Should a subject withdraw after administration of Eovist®, or should the investigators decide to withdraw the subject, all efforts will be made to complete and report the protocol-stipulated observations up to the time of withdrawal as thoroughly as possible. A final evaluation at the time of the subject's withdrawal should be made and an explanation given of why the subject is withdrawing or being withdrawn from the study. The reason and date and time of withdrawal must be recorded. If the reason for withdrawal is a clinical AE, monitoring will continue until the outcome is established.

3.9.1 Criteria for Removal from Protocol

- Investigators have the right to withdraw subjects from the study in the event of illness, AEs, or other reasons concerning the health or well-being of the subject, or in the case of lack of co-operation.
- Subject completes Eovist® enhanced MRI and has biopsy of the target lesion
- Patient requests to be taken off study
- Patient non-compliance with protocol guidelines

- Unacceptable treatment related toxicity as described in section **10.2.2**

Patient may not be taken off study until unacceptable toxicities have stabilized or resolved.

3.9.1.1 Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 CONCOMITANT MEDICATION/MEASURES

MRI contrast may be lessened by the following concomitant medications (OATP1B3 inhibitors)

- Select antibiotics (rifampicin, penicillin, ceftriaxone, cefmetazole, cefoperazone, and cefotaxime)
- Cyclosporin A
- Docetaxel / Paclitaxel
- Digoxin
- Fexofenadine
- Ibuprofen
- Imatinib
- Irinotecan
- Methotrexate

Any non-essential medications on the above list should be put 'on-hold' prior to the MRI for three days. Antibiotics should be finished before the MRI. If there is an essential medication that cannot be substituted then it will be noted but the patient will still be eligible for the study.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection

5.1.1 Clinical Data

All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.

Clinical data including summary and demographic data will be collected and entered into NCI CCR Database, C3D. Adverse events occurring during any scanning session will be recorded. Adverse events which are designated possibly, probably or definitely related to Eovist® will also be recorded.

Imaging data will include storage of the reconstructed images and image derived parameters on a secure, password protected lab imaging database. The lab imaging database will be stored

and maintained in the Molecular Imaging Program facilities. Personal identifiers will not be used when storing data.

Non-Anonymized images may also be stored in the clinical center PACS.

5.1.2 Safety Data

The following safety data will be collected and evaluated according to the Study Calendar (Section **3.6**):

- Clinical laboratory variables: serum biochemistry and hematology
- Injection site monitoring
- Pre-administration events (baseline signs and symptoms)
- AEs

SAEs will be recorded if they occurred as follows:

- After a subject first received Eovist® and throughout the subjects follow-up period,
- During the subject's follow up period, and for which a causal relationship to Eovist® cannot be ruled out.

Interpretation and follow-up of abnormal results will be done on a case by case basis in conjunction with the individual clinical situation. Any clinically significant abnormal finding, or change in one that represents a worsening from baseline, is an AE. Once a decision is reached to report a finding as an AE, the investigator is required to follow the procedure described for AE notification.

5.1.3 Imaging Data

Extracted imaging data include:

(1) All image data will be stored on a secure server, with access limited to credentialed users. This will permit flexible numeric raw data extraction for quantitative analysis and creation of summary data reports.

(2) Anonomymized image data from this study will be stored in a secure database that it is administered by the Cancer Imaging Program, NCI. The database is password protected and access is only given to qualifying collaborators.

5.2 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

6 BIOSPECIMEN COLLECTION

6.1 Tissue Samples

All tissue specimens (slide specimens) should be obtained as specified in the protocol and will be barcoded and labeled as described in the following sections. Tissue samples will be stained for immunohistochemistry analysis of OATP1B3 expression in NIH (please see section **3.5.3**).

In patients with localized disease, tissue can be obtained from image guided biopsies or from prostatectomy specimens.

In patients with advanced disease, tissue will be obtained from a target lesion (measuring ≥1.5cm in diameter at CT or MRI scan) by biopsy either incidental to this study or obtained specifically for this study.

6.2 Blood Samples

Blood samples will be collected for screening and on-study clinical laboratory safety assessments. All blood samples will be processed and handled in accordance with standard laboratory procedures. All samples except for the one obtained for OATP1B3 genotyping will be analyzed at the NCI Clinical Center laboratory. The peripheral blood sample obtained for OATP1B3 genotyping will be analyzed in Dr. William D. Figg`s Lab. Peripheral blood for DNA extraction will be collected in a 10-ml K2EDTA vacutainer. The vacutainer will be placed on wet ice after draw store in a 4°C refrigerator until pick-up. The research nurse (Yolanda McKinney) will record the date and time of draw on the blood tube label.

Please immediately page 102-11964 for pick-up. Contact the Clinical Pharmacology Program (CPP) processing group in 10/5A09 at 301-594-6131 or 301-402-3622 with any questions.

6.3 Pathology Specimens

6.3.1 Surgical Pathology

Specimens will be obtained from biopsy as described in Section **3.1.2** and mounted on glass slides. Slides will be labeled with study number, subject number, sampling date. Slide specimens will be stained to assess tumor tissue will be performed by one pathologist. IHC analysis will be conducted on slides to assess the expression of OATP1B3 (please see section **3.5.3**).

6.4 Sample Storage, Tracking and Disposition

6.4.1 Sample Processing and Storage:

Each patient research tissue sample will be assigned a unique patient identifier and relevant sample characteristics (such as timing of sample collection, treatment cycle and day identifiers) will be recorded. The location of all tissue samples will be carefully tracked in the secure database. All stored tissue samples will be coded and no identifying patient information will be on placed on sample containers. Stored tissue samples will be kept in freezers / refrigerators or secure containers located in the NCI research laboratories or in the laboratories of collaborators.

For the blood samples obtained for genotyping, peripheral blood samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Patient

Sample Data Management System (PSDMS) utilized by the CPP. This is a secure program, with access to PSDMS limited to defined CPP personnel, who are issued individual user accounts. Installation of PSDMS is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All CPP personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

PSDMS creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDMS access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in PSDMS. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6.4.2 Timeframe for research studies

Samples will be stored until requested by an authorized researcher(s). All researchers are required to use the samples for research purposes associated with this trial (as per the NCI IRB approved protocol). Subjects will be given the option of consenting to future use of their research samples per the informed consent process with their option declared in the consent document. Samples from those patients who consent to this will be stored permanently. However, these samples will be used only for research studies on active NCI IRB approved protocols covered by a valid informed consent document. Samples will be destroyed at the

completion of the study from those subjects who decline future use of their samples. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB approved protocol and patient consent. Any unused samples must be returned to the NCI laboratories as appropriate. The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (e.g. broken freezer or lack of dry ice in a shipping container) or if samples are destroyed because a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Any freezer problems, lost samples or other problems associated with samples will be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

7 STATISTICAL CONSIDERATIONS

7.1 Study Populations

25 evaluable patients will be included in this trial with an accrual rate of 1-2 patients a month. Therefore, it is expected that this study enrollment will be completed within 1.5 years and full study goals within 2.5 years. We plan to enroll 25 evaluable patients (10 in arm 1 and 15 in arm 2).

7.2 Subject Characteristics

Subject characteristics will be summarized with the following information:

- Number of subjects enrolled
- Number of subjects included in the safety analysis
- Number of subjects included in the efficacy analysis
- Number of subjects withdrawn from the study and the reason for withdrawal

Demographic information (age, height, weight, and body mass index) will be summarized using descriptive statistics. Race will be summarized by counts and percents.

Medical histories will be summarized by counts and percents. Concurrent medications will be recorded, coded and grouped by primary and secondary classes, if applicable.

7.3 Efficacy Analysis

7.3.1 Primary Efficacy Analysis

The primary objective of the study is to evaluate the uptake and retention of Eovist® in prostate cancers measured by the change of MRI parameter values between pre- and post-injection (section **3.5.1**). Log-transformed MRI parameter values between pre- and post-injection will be compared by the paired t-test. If the distribution of log-transformed MRI parameter values is skewed, nonparametric paired Wilcoxon signed-rank test will be used for comparison.

7.3.2 Secondary Efficacy Analysis

The secondary objective of the study is to evaluate Eovist® MR parameters with respect to OATP1B3 lesion expression.

Imaging will be performed on 2 subgroups of prostate cancer patients. Subgroup 1 consists of early stage (non-metastatic) patients, subgroup 2 late stage (confirmed metastatic with prior hormonal ablation therapy) patients. It is expected that OATP1B3 expression is higher in late stage patients (subgroup 2) than in early stage patients.

For patients in subgroup 2, expression of OATP1B3 will be determined in soft tissue or bone lesions taken from biopsy and will be correlated with MRI signal change by both Pearson and Spearman correlation coefficient.

As OATP1B3 SNPs also alter androgen uptake, affecting outcome of androgen deprivation therapy (ADT) [3, 4], genotype will also be compared to progression-free survival on ADT. Genotypes will be compared to these endpoints using the chi-squared and/or Fisher's exact test, or the log-rank test as appropriate.

7.4 Sample Size Determination

Sample size and power calculation is based on comparing signal change between pre- and post-injection in each subgroup separately. Based on a prior study on liver cancer patients, up to 3-fold mean signal change may be detected and the standard deviation of log-transformed signal ratio is approximately 0.25. With 10 evaluable patients in subgroup 2 (late stage), there is more than 95% power to detect a 2-fold mean signal change between pre- and post-injection at 5% significance level. For the first subgroup, OATP1B3 expression is likely lower and hence smaller signal change is expected. With 10 evaluable patients in each of these two subgroups, there is 83% power to detect a minimal 1.2 fold mean signal change. Higher power may be achieved if each patient has multiple lesions. Signal changes of multiple lesions from the sample patients will be averaged, and the comparison is based on the paired t-test using log-transformed signal values on a subject basis.

8 HUMAN SUBJECTS PROTECTION

8.1 Rationale for Subject Selection

The patient population in whom this disease occurs is adult males. All ethnic groups/ race categories would be represented as they are represented in the disease as a whole. Cognitively impaired individuals will not be included in this study if they are unable to understand the informed consent. Physically impaired persons who otherwise satisfy eligibility criteria will be included in this study. This study is considered more than minimal risk to subjects and no direct benefits to the patient are expected. We anticipate that a thorough discussion of the study at the time informed consent is obtained will minimize any susceptibility to undue influences and unnecessary risks to research subjects.

8.2 Participation of Children

Prostate cancer is not seen in children. Thus, we will not include children in this study.

8.3 Evaluation of Benefits and Risks/Discomforts

There is no possibility of direct benefit to participants in this study. The results of this trial could generate generalizable information that may benefit future patients with prostate cancer and therefore, subjects may benefit by knowing they have contributed to scientific knowledge.

The risks for participating are also minimal. Because the risks of Eovist® injection and MRI are uncommon, we anticipate a low rate of adverse events at the FDA-approved, low-dose level used in this study.

8.4 Risks/Benefits Analysis

Specific risks and potential complications will be clearly outlined in a separate consent form at the time of each procedure. Most complications are expected to be minor and require no treatment. Risks and discomforts associated with Eovist® injection and imaging are discomfort of an IV placement and the theoretical effects allergic reaction. The subject will be required to lay still on their back for back for 5-7 minutes for the MRI scan. The scan will give valuable information about the OATP1B3 status of prostate tumors and could lead to Improvements in prostate cancer therapies targeted to OATP1B3. Adults who are or may be unable to consent are excluded.

8.5 Consent and Assent Process and Documentation

The subject will be informed of the study by a member of the study team. Written and oral information about the study in a language understandable by the subject will be given to all subjects. The study will be explained in detail and the consent form and protocol (if requested) will be provided to the subject to take home (if desired) for consideration. If the subject has any questions they will be answered at the time of initial protocol discussion or later by telephone. Each subject's willingness to participate in the study will be documented in a signed and dated informed consent form before any procedures or assessments are done and after the aims, methods, anticipated benefits, potential hazards, and insurance arrangements in force are explained. It will also be explained to the subjects that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. The informed consent process will be documented in the subject's medical record and the investigator will sign, and date the informed consent form after the subject and/or legal representative has signed and dated it. The investigator(s) will keep the original consent forms and copies will be given to the subjects. Informed consent may be obtained from the patient by the PI, an associate PI or any clinical designee credentialed to obtain informed consent for any procedure.

9 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

9.1 Definitions

9.1.1 Adverse Events

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant requiring treatment. For this study, AEs will include events reported by the patient, as well as new onset abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or laboratory abnormality outside the range of normal limits or greater than 50% increase from baseline value if the baseline is above the upper limit of normal or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical Impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

9.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

9.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

9.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

9.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

9.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

9.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

9.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

9.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2 NCI-IRB Reporting

9.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations

- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

9.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

9.3 Data and Safety Monitoring Plan

9.3.1 Principal Investigator/Research Team

The Principal Investigator and Research Nurse will monitor the study for AEs.

This study will not have a formal data safety monitoring plan (DSMP), however, the Principal Investigator and Lead Associate Investigator will re-evaluate the protocol after each patient.

The principal investigator reserves the right to terminate the study on safety grounds. If 3 study subjects experience ≥ grade 2 Eovist®-related SAEs then the study will be terminated. Before terminating the study, the investigator will ensure that a review of the overall risk/benefit analysis confirms the balance to be no longer acceptable. Should termination be necessary, the investigator will arrange the relevant procedures, which will include informing the IRB, and other relevant local and national authorities. On termination of the study, the investigator will assure that adequate consideration is given to the protection of enrolled subjects' interests. Termination of the study will be considered in the event of significant safety findings occurring at any time during the performance of the study.

Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations and violations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10 PHARMACEUTICAL INFORMATION

10.1 Eovist® Source

Eovist® is an FDA approved pharmaceutical and it is commercially available from Bayer Pharmaceuticals, Inc.

10.2 Toxicity

10.2.1 Pharmacology and Toxicology in Animals

A dose-related increase in QT interval corrected for heart rate (QTc) which was resolved by 30 minutes post dosing was observed in dogs when given a single dose of Eovist®. The increase was noted when given in doses equal to or greater than 0.1 mmol/kg (2.2 times the human dose). Maximum increase in QTcF was equal to or less than 20 ms at doses up to 0.5 mmol/kg (11 times the human dose). A gait disturbance was observed in 1 of 3 mice when given Eovist® at a dose of approximately 1.1 mmol/kg (3.6 times the human dose); the disturbance occurred at 30 minutes post dosing and resolved at 4 hours post dosing.

10.2.2 Toxicity in Humans

Toxicities are not anticipated in this study. In the event that a patient experiences an allergic reaction, or toxicity greater than grade 2 during the Eovist® injection, the injection will be stopped immediately and standard supportive measures instituted. No further Eovist® will be administered.

Overall, 4.3% of subjects reported one or more adverse reactions during a follow-up period that for most subjects, extended 24 hours after Eovist® administration. The reported AEs of Eovist® were feeling hot (0.9%), nausea (0.6%), headache (0.5%), injection site reactions, the reported serious reaction rate is less than 0.5% [6].

Gadolinium-based contrast agents (GBCAs) increase the risk for nephrogenic systemic fibrosis (NSF) among patients with impaired elimination of the drugs. The GBCA-associated NSF risk appears highest for patients with chronic, severe kidney disease (GFR <30 mL/min/1.73m^2) as well as patients with acute kidney injury. The risk appears lower for patients with chronic, moderate kidney disease (GFR 30–59 mL/min/1.73m^2) and little, if any, for patients with chronic, mild kidney disease (GFR 60-89 mL/min/1.73m^2). NSF may result in fatal or debilitating fibrosis affecting the skin, muscle and internal organs [6].

Anaphylactoid and anaphylactic reactions with cardiovascular, respiratory and cutaneous manifestations, ranging from mild to severe reactions, including shock have uncommonly occurred following Eovist® administration. Most hypersensitivity reactions to Eovist® have occurred within half an hour after administration. Delayed reactions (hours up to several days) may occur [6].

10.2.3 Formulation and Preparation

Eovist® contains the active pharmaceutical ingredient gadoxetate disodium (Gd-EOB-DTPA), which is designated chemically as (4S)-4-(4-Ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9-triazaundecanedioic acid, gadolinium complex, disodium salt with a molecular weight of 725.72 and an empirical formula of GdC23H28N3O11Na2.

Each mL of Eovist® contains 181.43 mg of gadoxetate disodium (equivalent to 0.25 mol/L gadoxetate disodium) and the excipients caloxetate trisodium, trometamol, hydrochloric acid and/or sodium hydroxide (for pH adjustment), and water for injection. Eovist® contains no antimicrobial preservative. Eovist® has a pH of 6.8 to 8.

10.2.4 Stability and Storage

The intact vials remain clinically acceptable until the expiration date indicated on the vial. Eovist® should be stored at temperatures between 20–25°C (68–77°F); excursions permitted to 15–30° C.

10.2.5 Administration Procedures

Eovist® is usually directly injected intravenous through an intravenous line at a rate of 2 mL/second.

10.2.5.1 Supply and Packaging

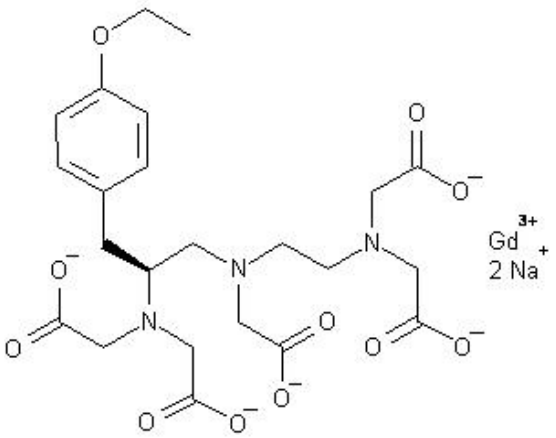
Eovist® is supplied in single-dose, rubber stoppered vials containing 181.43 mg/mL of gadoxetate disodium, equivalent to 0.25mmol/mL, in the following sizes: 10 mL single-dose vials filled with 10 mL, in individual cartons, boxes of 20 NDC 50419-320-01. No additional preparation is necessary.

10.2.6 Incompatibilities

Anionic drugs primarily secreted into the bile (such as rifampicin) may reduce the hepatic contrast enhancement and the biliary excretion of Eovist®.

The chemical structure of Eovist® is shown in Figure 2 below.

Figure 2 Structure of Eovist®



Molecular formula: $GdC_{23}H_{28}N_3O_{11}Na_2$

Molecular weight: 725.72

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INSTITUTE: National Cancer Institute

STUDY NUMBER: 13-C-0145 PRINCIPAL INVESTIGATOR: Ismail Baris Turkbey, M.D.

STUDY TITLE: Pilot Study of Eovist® (Gadoxetate) Enhanced MRI for the Detection of Prostate Cancer

Continuing Review Approved by the IRB on 01/12/15

Amendment Approved by the IRB on 05/12/15 (D) Date Posted to Web: 05/13/15

Standard

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

Prostate cancer is the most common cancer type among men. Although some prostate cancers respond to hormonal therapy, there are cellular characteristics of other prostate cancers that cause it not to respond as well to hormonal therapy. The purpose of this study is to test Eovist, an imaging agent, to see if it is helpful in identifying these different types of prostate cancers using MRI scans.

Eovist (gadoxetate disodium) is a magnetic resonance imaging (MRI) contrast agent that was

approved by the FDA to use in MRI of the liver to detect and characterize lesions (damages in tissues) in adults with known or suspected liver disease. Eovist's uptake in liver lesions is recently shown to be linked with presence of a biomarker called OATP1B3. A biomarker is molecule that can be used as an indicator of a particular disease. This biomarker is an abundant molecule in prostate cancers which are resistant to hormonal therapy. So use of Eovist to detect the presence of OATP1B3 in the prostate cancer in this protocol is experimental. As a long term scientific goal we hope to find out if Eovist will be predictive for hormonal therapy resistance.

Why are you being asked to take part in this study?
You are being invited to take part in this research study because you have prostate cancer and will have surgery to remove your prostate or will have biopsy of your tumor which is located outside your prostate.

How many people will take part in this study?
Up to 25 people will be included in this study.

Description of Research Study

What will happen if you take part in this research study?
Before you sign this document, you will have several procedures to determine if you are eligible for this study. Several vials of blood will be drawn for a panel of tests. You will also have a physical exam and answer questions about your medical history and current medications. Your doctor will review all the eligibility requirements to ensure you meet all the criteria before you enter the study. If you are determined to be eligible for this study, you will then be scheduled to return for the study injection and imaging scans.

Imaging Studies (Scans):

MRI Imaging:

A standard MRI sensor, which is like a small blanket with wiring inside, will be wrapped around your lower torso to improve MRI quality. The MRI scan usually takes less than an hour. You will need to lie still on the scanning table during that time. An intravenous line (IV; a very thin catheter inserted into a vein with a tiny needle) will be placed in your arm before your MRI scan. The agent called Eovist will be injected into your arm through the IV. The MRI imaging will be performed just before the Eovist agent is injected into your arm. MRI imaging will also be performed 10, 20 and 60 minutes after the Eovist agent is injected into your arm. You will receive more information about your MRI scan when you visit the Molecular Imaging Clinic. You may ask questions at any time. In addition, you should ask your doctor or the study doctor any questions you have concerning this study.

Biopsy:
If you have undergone surgery, your prostate will be studied for tumor cells. This biopsy procedure will be standard of care. If you do not undergo surgery, some tissue from one of your

tumors located outside the prostate gland may be removed by a needle if that tumor has not been biopsied before. This biopsy procedure will not be performed as standard of care but as a separate additional research biopsy.

The tissue collected during your biopsy will be evaluated at NIH just as it would be if you were not participating in this trial. Your tissue will be evaluated for biomarkers that predict uptake of Eovist by your tumor.

Birth Control

If your partner can become pregnant, you or your partner will need to practice an effective form of birth control during the time you are participating in this protocol. If you think that your partner is pregnant, you should tell your study doctor or nurse at once.

Effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- hormonal (birth control pills, injections, or implants)
- tubal ligation
- vasectomy

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

This is not a treatment trial. Instead of being in this study, you have the following options:

- Not participating in this study or
- Taking part in another study

Risks or Discomforts of Participation

Risks of MRI

Having an MRI requires that you lie still with part of you or all of you inside a tube shaped machine for about 45 minutes to an hour. Even with the ear plugs we give you it can be noisy with loud clicking and thumping sounds, which bothers some people. Some people may feel 'closed in' or 'trapped' (even though they being closely watched and are quite safe). This is called claustrophobia. Cool air will surround you, and the room is large and brightly lit to help avoid claustrophobia. You may ask your physician for a mild sedative for the procedure if you think it will help. If you take a sedative you must not drive a vehicle until it wears off after the MRI.

MRI scans cannot be done on people who have:

- a cardiac pacemaker,
- neural pacemaker,
- surgical metal clips in the brain or on blood vessels,
- cochlear implants,
- or foreign metal objects within the eye.

At the time of your MRI, you will be asked about these things.

Risks from Eovist

Eovist has been generally safe when given to people for its FDA approved for imaging of liver disease. Side effects of Eovist may occur in this trial. If they do happen it is usually right around the time that Eovist is injected, which is why you will be closely watched at that time, and promptly treated if necessary. These effects include:

- whole body allergic reactions, which can range from mild to serious or life threatening (this is called anaphylaxis),
- low blood pressure, which can lead to becoming unconscious (fainting, passing-out)
- feeling hot, nausea, headache

Risks from Biopsy

Biopsies are normally performed under the guidance of an imaging technique. Each procedure requires a separate consent prior to the biopsy. The risks may include:

- Pain and discomfort. The amount of pain and discomfort will vary, depending on the location of the biopsy site. These risks can be discussed with the study doctor.
- Minor bleeding at the biopsy site.
- Tenderness at the biopsy site.
- Scarring at the biopsy site.
- Rarely, an infection at the biopsy site.

Uncommonly, complications from biopsies can be life threatening. As with any interventional procedure, other potentially serious complications from bleeding or organ damage may occur. These might require additional surgical intervention.

Radiation Exposure:

This research study involves exposure to radiation from CT guided biopsy (the effective dose from one CT guided biopsy is 0.15rem). This radiation exposure is not required for your medical

care and is for research purposes only. The amount of radiation you will receive in this study is below the guideline of 5 rem (or 0.5 rem in children) per year allowed for research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. If you would like more information about radiation, please ask the investigator for a copy of the pamphlet, An Introduction to Radiation for NIH Research Subjects.

While there is no direct evidence that the amount of exposure received from participating in this study is harmful, there is indirect evidence it may not be completely safe. There may be a very slight increase in the risk of cancer.

Please tell your doctor if you have had any radiation exposure in the past year, either from other research studies or from medical tests or care, so we can make sure that you will not receive too much radiation. Radiation exposure includes x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into your body.

Potential Benefits of Participation

Are there benefits to taking part in this study?

There is no direct benefit to participating in this study. However, the knowledge gained from this study may help others in the future.

Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Participation in the study

Your doctor may decide to stop your participation for the following reasons:

- if he/she believes that it is in your best interest
- if you have side effects from the procedures that your doctor thinks are too severe

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. The following link contains details on this process *http://ethics.od.nih.gov/procedures/COI-Protocol-Review-Guide.pdf*. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

Optional Studies

We would like to keep some of the specimens and data that are collected for future research. These specimens and data will be identified by a number and not your name. The use of your specimens and data will be for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you decide now that your specimens and data can be kept for research, you can change your mind at any time. Just contact us and let us know that you do not want us to use your specimens and/or data. Then any specimens that remain will be destroyed and your data will not be used for future research.

Please read each sentence below and think about your choice. After reading each sentence, circle and initial the answer that is right for you. No matter what you decide to do, it will not affect your care.

1. My specimens and data may be kept for use in research to learn about, prevent, or treat cancer or other health problems.

Yes No Initials_____

2. Someone may contact me in the future to ask permission to use my specimens and/or data in new research not included in this consent.

Yes No Initials_____

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Ismail Baris Turkbey, M.D., Building 10, Room B3B85, Telephone: 301-443-2315. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM JANUARY 12, 2015 THROUGH JANUARY 11, 2016.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____