

Contrast-enhanced Ultrasound for Complex Kidney Lesion Diagnosis in Patients with CKD extension (CEUS CKDx)

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CEUS CKDx

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CONFIDENTIAL
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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_____

PI Signature: _____

Date:_____

TABLE OF CONTENTS

1.0 BACKGROUND AND RATIONALE	5
1.1 Study Synopsis	5
1.2 Background	5
1.3 Perflutren Lipid Background and Associated Known Toxicities.....	7
1.4 Rationale.....	10
2.0 STUDY OBJECTIVES.....	10
2.1 Primary Objective	10
2.2 Secondary Objectives.....	10
3.0 PATIENT ELIGIBILITY	10
3.1 Inclusion Criteria.....	10
3.2 Exclusion Criteria.....	11
4.0 STUDY PLAN	12
4.1 Schema	12
4.2 Study Design	13
4.3 Follow-up	15
4.4 Duration of Study Intervention	15
4.5 Duration of Follow Up	16
4.6 Removal of Patients from Protocol	16
5.0 DRUG INFORMATION	16
5.1 Perflutren Lipid Microspheres (Lantheus Medical Imaging).....	16
5.2 Supplier/How Supplied	16
5.3 Handling and Dispensing of Perflutren.....	16
5.4 Storage Requirements/Stability	17
5.5 Preparation	17
5.6 Dosage and Administration	17
5.7 Clinical Safety Summary	17
5.8 Return and Retention of Study Drug.....	18
6.0 EVALUATIONS AND ASSESSMENTS.....	18
6.1 Pre-Study Assessments	18
6.2 Treatment Assessments	19
6.3 Follow-up Assessments.....	19
6.4 Assessment of Safety	19
6.5 Tissue Collection and Optional Storage.....	19
7.0 ADVERSE EVENTS	19
7.1 Definitions	19
7.2 Documentation of non-serious AEs or SARs.....	21

7.3 SAEs or Serious SARs	21
7.4 Data and Safety Monitoring Plan	22
8.0 STATISTICAL CONSIDERATIONS	24
8.1 Study Design/ Study Endpoints	24
8.2 Sample Size and Accrual.....	24
8.3 Data Analysis Plans.....	24
9.0 STUDY MANAGEMENT	25
9.1 Institutional Review Board (IRB) Approval and Consent.....	25
9.2 Required Documentation.....	26
9.3 Data Management and Monitoring/Auditing	26
9.4 Adherence to the Protocol	27
9.5 Amendments to the Protocol	28
9.6 Record Retention	28
9.7 Obligations of Investigators	29
10.0 REFERENCES.....	29
11.0 APPENDICES	32

1.0 BACKGROUND AND RATIONALE [L] [SEP]

1.1 Study Synopsis [L] [SEP]

This is an investigator-initiated, prospective study designed to evaluate the accuracy of contrast-enhanced ultrasound (CEUS) with microbubble contrast agent (perflutren lipid; Definity®) to identify malignancy in patients with kidney disease, a known risk factor for kidney malignancy, in whom a conventional ultrasound (US) or other prior imaging shows an indeterminate or suspicious kidney lesion. [L]
[SEP]

The primary objective of the study is to estimate the sensitivity of CEUS in diagnosing kidney malignancy in patients with a risk factor for kidney malignancy and compare it to the current truth standard test in this patient population. Secondary analysis will include more optimal contrast-enhanced imaging with computed tomography (CT) or magnetic resonance imaging (MRI) in a subset of patients who can receive these studies. The study outcome will lead to immediate clinical application in patients with chronic kidney disease. Given the cost-effectiveness and adverse event profile, it has excellent potential to become established as first line diagnostics in the general patient population as well.

1.2 Background [L] [SEP]

Introduction [L] [SEP]

According to the US Renal Data System 2014 report, there are over 600,000 people in the US living with end stage kidney disease (ESKD) with almost 450,000 receiving dialysis. In a large collaborative international study, kidney cancers have a standardized incidence ratio of 3.6 in patients receiving dialysis¹ meaning that for the nearly 500,000 patients receiving dialysis, there are an additional 50,000 cases of kidney cancer that occur above that of the general population. More recent findings show that patients with chronic kidney disease (CKD) are also at increased risk of kidney cancer, with hazard ratios increasing with CKD stage². For this reason, patients on the waiting list for renal transplant and those receiving dialysis have been advised to have annual screenings for kidney malignancy.

Currently, contrast-enhanced CT and MRI are the truth standard imaging studies for kidney lesion characterization. However iodinated contrast agents are nephrotoxic in patients with renal insufficiency³ and therefore contraindicated in patients with moderate to severe CKD (stages IV and V) as they can lead to permanent deterioration of kidney function. Until recently, contrast MRI was a suitable alternative. However, Nephrogenic Systemic Fibrosis (NSF) is now regarded as a serious, potentially lethal complication of the gadolinium contrast agents used in MRI if administered to patients with impaired renal function. Inadequate preoperative imaging can lead to unnecessary nephrectomy, which would further limit already compromised renal function⁴. There is therefore a need for an imaging

method for evaluating renal masses that does not use iodinated or gadolinium contrast agents. CEUS represents one potential alternative.

Contrast-Enhanced US Diagnostic Imaging of the Kidney^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24}

For CKD patients screened annually for kidney malignancy, US imaging of the kidney is typically performed in the United States without contrast as no US contrast agent is approved for this indication. Without contrast, malignant tumors cannot be differentiated from benign tumors or pseudotumors. Typically, following the identification of a kidney lesion via US without contrast, contrast-CT or MRI may then be performed to stage and grade the lesion, depending on a risk/benefit analysis performed by the patient's physician. If a contrast-CT or MRI is deemed too risky, the patient may proceed directly to surgery, or more likely, will be followed closely with repeated non-contrast imaging, typically anywhere from 1-5 years.

US contrast agents are gas-filled microbubbles which are intravenously administered in very small volume boluses or slow infusions. These microbubbles typically have mean diameters between 1 to 6 μm ⁵, remain intravascular for several minutes, and do not diffuse into the interstitium (pure blood-pool agents)⁵.

Advancements in imaging technique combined with the nonlinear properties of microbubbles have spurred the development of contrast-specific US techniques such as CPS (Contrast Pulse Sequencing). Such imaging techniques provide high-resolution images of tissue vasculature and allow the assessment of the microcirculation patterns in real-time⁶⁻¹². Outside of the United States, or in the context of clinical trials, US contrast agents have been used in kidney imaging^{8-11, 13-20}. Levovist® (lipid and galactose microparticle suspension) and SonoVue® (lyophilized sulfur hexafluoride microbubbles), two non-FDA approved similar US contrast agents approved for use in countries outside of the United States, have been used in the general population to identify and diagnose renal pseudotumors^{10, 18, 21}, cystic renal lesions^{10, 14, 16, 18, 22} and solid renal lesions^{10-13, 15, 17-19}. See a descriptive table of these studies below. Two recent studies have included patients with CKD and shown similar accuracy in this population.^{23, 24}

Author (year)	Contrast Agent	Imaging Method	# lesions	Lesion Type	Results
Kim (1999) ¹⁴	Levovist®	Power Doppler	13	Cystic	PPV: 100% NPV: 57% DxA: 77%
Ascenti (2001) ¹³	Levovist®	Power Doppler	41	Solid	DxA: 78%
Ascenti (2004) ¹⁵	SonoVue®	2 nd Harmonic	40	Solid	Se: 85.7%
Park (2005) ¹⁷	Levovist®	2 nd Harmonic	30	Solid	Se: 97% Sp: 93% DxA: 95%

Tamai (2005) ¹⁹	Levovist®	SieFlow	29	Solid	Se: 94.4% Sp: 45.4% PPV: 100%
Wink (2007) ⁸	SonoVue®	CPS	20	Solid & Cystic	Se: 95%
Quaia (2008) ⁹	SonoVue®	CPS	40	Cystic	Se: 89% Sp: 73.6% PPV: 79.3% NPV: 86% DxA: 82%
Fan (2008) ¹²	SonoVue®	CPS	72	Solid	Sp: 96.4% Se: 77.3%
Meloni (2008) ¹¹	SonoVue®	CPS	30	Solid	Se: 96.6% Sp: 100% PPV: 100% NPV: 95.8% DxA: 98.1%
Roy (2008) ¹⁸	SonoVue®	CPS	86	Solid & Cystic	Sp: 92.9% - Papillary carcinoma 57.1% - Clear Cell carcinoma 100% - Oncocytoma 100% - Benign
Abbreviations: PPV – Positive Predictive Value, NPV – Negative Predictive Value, DxA – Diagnostic Accuracy, Se – Sensitivity, Sp – Specificity					

In the United States, where there has been significantly less research on using US contrast in humans, the US contrast agent, Definity®, has recently been used in one study to measure renal blood flow in a healthy population²⁰, effectively reproducing previous animal studies in the human population. Three US contrast agents are FDA approved for human use: perflutren lipid microspheres (Definity®), sulfur hexafluoride lipid microspheres (Lumason®) and perflutren protein-type A microspheres (Optison®). All are FDA indicated for use in cardiac studies, and Lumason® is also FDA approved for liver and urinary tract imaging.

1.3 Perflutren Lipid Background and Associated Known Toxicities

See <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/> for full prescribing information on perflutren when used according to its FDA-approved indication. Also see section 5.7.

1.3.1 Background

Definity® (perflutren lipid) is an FDA-approved US contrast agent indicated to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border in patients with sub-optimal echocardiograms. It is activated by mechanical agitation with a Vialmix® which produces a milky white injectable suspension of perflutren lipid microspheres composed of octafluoropropane. Activated perflutren may be injected by either an intravenous bolus or infusion. See <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/> for full prescribing information when used in this indication. When used in this setting, the maximum dose of perflutren is administered as either two bolus doses or one single intravenous infusion.

1.3.2 Associated Toxicities

In pre-market clinical trials, 1716 subjects were evaluated with activated perflutren lipid. Of the 1716 subjects, 144 (8.4%) had at least one treatment-related adverse reaction. There were 26 serious adverse events and 15 (0.9%) subjects discontinued because of an adverse event. Nineteen subjects (1.1%) suffered serious cardiopulmonary adverse events including 8 deaths. The deaths occurred several days after activated perflutren lipid administration and appear to be related to the course of underlying disease. Of the 11 other serious adverse events, which appeared within 2-15 days of the drug administration, all appeared to be a progression of underlying cardiac and non-cardiac disease. However, a role for perflutren lipid in the initiation or course of these adverse events cannot be ruled out.

There were 15 discontinuations reported. Nine of these patients were discontinued after the first injection. One patient experienced a hypersensitivity reaction with urticaria and pruritus and all the other patients experienced dizziness, chest pain, dyspnea or back pain. Adverse events (AEs) appeared within 1 – 15 minutes of the drug administration and were of moderate intensity resolving usually without treatment within minutes or hours after onset.

For all AEs, there were no differences in the overall incidence based on age, gender, or route of administration. The most common events were (% of patients experiencing): headache (2.3%), back and renal pain (1.2%), flushing (1.1%) and nausea (1.0%).

Cardiopulmonary Reactions^{[11][SEP]}

In 2007, in response to post-marketing reports of 4 deaths and 190 serious cardiopulmonary reactions, the FDA issued a black box warning for both Definity® and Optison® adding disease state contraindications and a mandatory 30 minute monitoring period following administration in all patients. Following this there have been several large-scale safety studies looking into the records of a total of more than 200,000 patients who received one of these contrast agents. In all those studies a composite rate of serious adverse events was calculated to be 1 – 3 in 10,000,²⁵ compared to gadolinium-based MRI contrast which has an incidence of NSF of 2 – 5 in 100 patients with chronic kidney disease.²⁶

Following a meeting of the FDA Cardio-renal Advisory Committee in 2008, the black box warning was revised. The revisions shortened the contraindications to include cardiac shunts and hypersensitivity to perflutren and mandated the 30-minute monitoring period be limited to patients with pulmonary hypertension or unstable cardiopulmonary conditions. The black box warning was further revised in 2011, removing the mandatory 30-minute monitoring period, but stating that most serious cardiopulmonary reactions occur within 30 minutes of administration. For this reason, the label states that cardiopulmonary resuscitation personnel and equipment be readily available prior to perflutren administration, and that all

patients be monitored for acute reactions.

Patients with a history of cardiac shunts, pulmonary hypertension or unstable cardiopulmonary conditions will be excluded from our study. In addition, all patients will be monitored for 30-minute post-perflutren administration by the research or clinical nurse or research physician.

Hypersensitivity Reactions^{[1][2]}_{SEP}

The real risk of perflutren in our study is to the small number of potential patients with an undiagnosed allergy to perflutren. Post-marketing reports have included anaphylactoid events and other serious but non-fatal adverse reactions, typically within 30 minutes of drug administration (see the package insert, and section 5.7 for additional information. In order to avoid a potentially fatal event, EpiPen® (epinephrine) injections will be kept near the US machine for all patients.

High Ultrasound Mechanical Index (MI)^{[1][2]}_{SEP}

High ultrasound MI values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. In addition, end-systolic triggering with high MI has been reported to cause ventricular arrhythmias. While the safety of perflutren at MIs >0.8 has not been established by the manufacturer, real-time measurement of renal blood flow in 19 healthy subjects using perflutren was recently reported, using a flash replenishment high-MI (MI of 1.0) US technique. The contrast agent was well tolerated with no serious adverse events. One patient each had a 20 mmHg increase in systolic blood pressure, a very brief and mild episode of flushing, and mild back pain, but no cardiac arrhythmias were recorded during the study period.²⁰

A second study using the Sonovue® contrast agent with flash-replenishment high-MI to detect changes in kidney perfusion via US in 10 normal volunteers reported the agent was well tolerated, and not associated with any adverse events.²⁷ The safety of using a flash-replenishment high MI echocardiography with Sonovue®²⁸ has been established in patients with chest pain of undetermined origin as well as in patients undergoing stress testing for detection of ischemia.²⁹

Use in Renal Insufficiency^{[1][2]}_{SEP}

US contrast agents including perflutren should carry no additional risks in patients with renal insufficiency, as perflutren is cleared by the lungs. The phospholipid component of perflutren lipid microspheres is thought to be metabolized to free fatty acids, while the octafluoropropane (OFP), as a stable gas, is not metabolized. In a small (n=8) pharmacokinetic study in healthy subjects, OFP was undetectable after 10 minutes in most subjects either in the blood or expired air, with a mean half-life of 1.3 minutes (Definity® Prescribing Information).

The UNC Division of Cardiology routinely uses perflutren as an echocardiographic contrast agent, including in patients with renal insufficiency.

1.4 Rationale

At this time, contrast-enhanced CT and MRI are the standard imaging studies for evaluation of suspected renal tumors. However, there are serious adverse effects from iodinated CT contrast and gadolinium MRI contrast in patients with renal insufficiency. Currently, to reduce exposure of potentially nephrotoxic contrast agents in patients with renal insufficiency, use of iodinated CT and gadolinium MR is limited to those patients in whom US without contrast indicates a suspicious or indeterminate lesion, and for whom the risk/benefit ratio warrants this risk.

Our primary objective is to compare the accuracy of perflutren enhanced US to the current clinical truth standard of follow-up non-contrasted imaging in patients with CKD. The accuracy of perflutren-enhanced US will be determined by comparing results of the perflutren-enhanced US with the Truth Standard (tissue diagnosis or change in 1-year follow-up non-contrast US imaging). In patients where contrasted imaging (CT or MRI) is available, these results will be incorporated into the Truth Standard in a secondary analysis.

Unlike CT and MRI contrast agents, US contrast agents are not associated with complications in patients with impaired kidney function. We hope to show that contrast-enhanced US may provide a viable alternative to contrast-enhanced CT and MRI for identifying malignancy in kidney lesions in patients with impaired kidney function in view of the serious adverse effects associated with CT and MR contrast agents in this patient population.

2.0 STUDY OBJECTIVES**2.1 Primary Objective**

To estimate the sensitivity and specificity of qualitative interpretations of CEUS in diagnosing kidney malignancy in patients with CKD and a suspicious or indeterminate lesion on non-contrasted imaging compared to the truth standard described in 1.4 and 4.2.6.

2.2 Secondary Objectives

To compare sensitivity and specificity of qualitative interpretations to contrast-CT or MR in a subset of patients.

To compare sensitivity and specificity of two different scoring/classification systems (Bosniak and Barr 2014) for use in CEUS of kidney lesions in patients with CKD.

3.0 PATIENT ELIGIBILITY**3.1 Inclusion Criteria**

To be eligible for the present study, patients must meet the following criteria: [L] [SEP]

3.1.1 Able to provide written informed consent [L] [SEP]

3.1.2 Willing to comply with protocol requirements [L] [SEP]

3.1.3 At least 18 years of age [L] [SEP]

3.1.4 Have kidney disease, defined as either CKD II-V, determined by estimated glomerular filtration rate (GFR) of <90 and derived from serum creatinine measurements, or albuminuria/proteinuria, determined by albumin to creatinine ratio or protein to creatinine ratio of >30mg/gm within 3 months of recruitment, or on dialysis or having received a kidney transplant or have biopsy proven kidney disease. In these latter cases, blood and urine tests are not necessary.

3.1.5 Have at least one kidney lesion identified but incompletely characterized on a non-contrasted US, CT, or MR exam for which the patient's provider recommends follow-up studies or further evaluation with additional imaging test(s).

3.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded for enrollment:

3.2.1 Critically ill or medically unstable and whose critical course during the observation period would be unpredictable (e.g., chronic obstructive pulmonary disease (COPD) requiring oxygen) [L] [SEP]

3.2.2 Known hypersensitivity to sulfur hexafluoride or to any component of perflutren lipid (Definity®) [L] [SEP]

3.2.3 Right to left shunt, severe pulmonary hypertension (Pulmonary artery pressure >90mmHg), or adult respiratory distress syndrome [L] [SEP]

3.2.4 Active cardiac disease including any of the following:

- Severe congestive heart failure (class IV in accordance with the [L] [SEP]classification of the New York Heart Association) [L] [SEP]
- Unstable angina. [L] [SEP]
- Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; [L] [SEP]ventricular premature complexes occurring close to the preceding T-[L] [SEP]wave, multifocal complexes). [L] [SEP]
- Myocardial infarction within 14 days prior to the date of proposed Definity® administration. [L] [SEP]
- Uncontrolled systemic hypertension (systolic blood pressure (BP)>180 mm Hg and/or diastolic BP>100 mm Hg despite optimal medical management) [L] [SEP]

3.2.5 Is in an intensive care setting ^[L]_[SEP]

3.2.6 Has an unstable neurological disease (e.g cerebrovascular accident (including transient ischemic attacks (TIAs) within the 3 months before signing of informed consent

3.2.7 Has undergone an invasive procedure on kidney lesion (e.g. tissue biopsy, surgery, nonsurgical cytoreductive procedure) since identification of lesion via US without contrast

3.2.8 Has any other medical condition or other circumstances that would significantly decrease the chances of obtaining reliable data or of achieving the study objectives such as: ^[L]_[SEP]

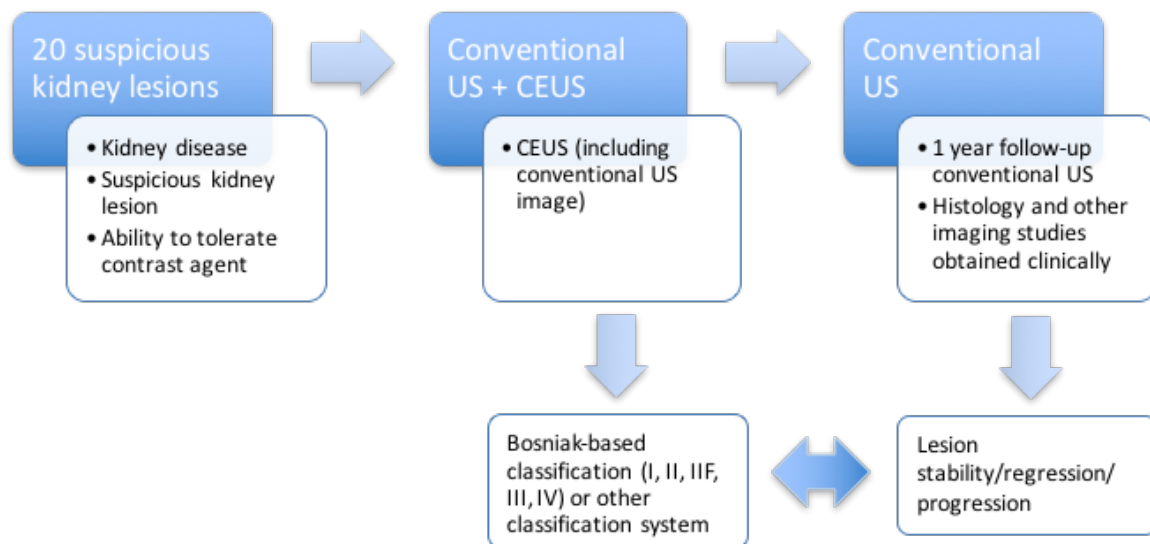
- Mental illness
- Drug abuse ^[L]_[SEP]

3.2.9 Female patient who is pregnant or lactating (the possibility of pregnancy has to be excluded by negative POC, serum or urine B-HCG results, obtained within 24 hours before the perflutren lipid administration, or on the basis of patient history, e.g.: tubal ligation, hysterectomy or a minimum of 1 year without menses)

3.2.10 Obesity that limits obtainment of acceptable images

4.0 STUDY PLAN ^[L]_[SEP]

4.1 Schema



Patients who meet study inclusion and exclusion criteria (see Sections 3.1 and 3.2)

and provide informed consent will be subjected to a baseline conventional, non-contrasted lesion-focused ultrasound on Day 1 and CEUS. One year after initial study, a follow-up non-contrasted ultrasound will be obtained. The results of any other additional studies obtained clinically, including histology, contrasted or non-contrasted CT/MR, will be collected. Once all CEUS studies are completed, blinded radiologists will grade each lesion based on Bosniak criteria. Once all follow-up non-contrasted ultrasounds are obtained, radiologists will grade each lesion as stable, progressed, or regressed.

Please see the Time and Events Table in Appendix 2 for a full listing of evaluations and assessments throughout the course of the study.

4.2 Study Design

4.2.1 Patient Identification and Recruitment

Patients will be identified through UNC's nephrology, transplant and dialysis clinics. Additionally, community collaborators will be alerted to the study and asked for referrals. Patients who participated in a prior pilot study (IRB 12-2314) with similar inclusion and exclusion criteria will be screened to determine if they still meet criteria. The PI will communicate with the patient's referring clinician and the patient to determine whether they fit the inclusion/exclusion criteria and would like to participate in the study. The study coordinator will then be notified of these patients and approach the patient to further explain the study and obtain their signature on the informed consent form if they wish to participate. If patient consents, they will undergo perflutren-enhanced US. We will provide \$50 compensation and a parking voucher for completion of initial CEUS study. If two lesions are identified and imaged, either the day of the initial study visit or return visit, they will receive \$50.00 for each lesion imaged. Individuals will also be compensated an additional \$50.00 if a lesion needs to be reimaged on a return visit. We will offer an additional \$50 for completion of all follow-up studies (1-year urinalysis and serum creatinine and 1-year follow-up conventional ultrasound or other imaging study) and \$75 if they are not cared for at UNC and need to make an additional trip to UNC to obtain the studies. We plan on enrolling a total of 20 patients that fit the above criteria over a 6-month period.

Healthy volunteers will also be recruited at study initiation to optimize the imaging technique. These volunteers will receive equivalent compensation. In the case of any abnormalities found during the study, the subject will be informed of the finding, and the finding will be communicated to the subject's primary provider by study PI for further management. Imaging optimization is needed to optimize the method of the administration of Definity. A total of 5 health volunteers will be included in the imaging optimization phase.

Patients that decline enrollment will be asked if they would be willing to participate

as controls. This would involve no CEUS study and only collection of serum creatinine levels over a 1-year follow-up period from the patient's primary provider. These patients will be consented by the study coordinator to allow patients to be followed.

4.2.2 Multicenter sites.

This will be a single institution study.

4.2.3 Sonographic Evaluation

Study subjects will be asked to lie on an examination table in the UNC Radiology Department of US. Once the patient is positioned, US gel will be applied to the patient's back or flank. The transducer will be placed on top of the gel. Once the kidney lesion is located, the distance from the transducer to the nearest edge of the target lesion will be recorded. Pre-contrast Doppler and B-mode investigations of the target lesion will be performed at baseline using commercially available US equipment and standard technique. Subsequent to this, either color or power Doppler imaging should be used to study the anatomy of the target lesion and surrounding vessels. Images (baseline and perflutren-enhanced) will be digitally recorded on a cine loop.

4.2.4 Perflutren Administration ^[1]_{SEP}

See <http://www.definityimaging.com/pdf/VIALMIX%20Users%20Guide.pdf>, and the package insert, for instructions on perflutren lipid preparation and activation. Perflutren lipid is intended for intravenous (IV) administration only after activation in the Vialmix® apparatus.

Resuscitative carts and trained personnel will be immediately available during Perflutren administration and imaging.

Perflutren will be administered in a bolus or continuous infusion using the dosing range and administration type within the perflutren prescribing information (see <http://www.definityimaging.com/how-administration.html>). If a subject has a central port 20 gauge or greater and does not have a filter then perflutren will be administered through the central port. If a subject does not have a central port then perflutren will be administered through the antecubital vein, preferably on their dominant hand.

Once perflutren lipid has been administered, the transducer is maintained in a constant position over the area of interest to show the target lesion in order to assess the enhancement pattern during the early, mid and late vascular phases. Either bolus or infusion imaging with flash replenishment imaging approaches will be used with MI not to exceed 1.1. Images will also be taken of kidney parenchyma in a suitable longitudinal plane. Imaging with perflutren lipid will be performed with software (Siemens Medical Solutions USA, Inc) using the flash-replenishment high-

MI imaging technique for parenchymal imaging.

The contrast-enhanced US examination will consist of the complete dynamic real-time assessment of the contrast enhancement profile of the lesion in comparison to the surrounding renal parenchyma. If there are multiple lesions in one subject requiring a second dose, the subject will have the option to undergo a 2nd contrast-enhanced study 30-minutes after the initial contrast dose, per dosing instructions in the package insert. Individuals may also be asked to return on a different study date to reimage a lesion or to image additional lesions.

Upon completion of imaging study, the patient will be brought back to the radiology holding area and monitored by nursing staff for 30 minutes after study completion. Blood pressure and heart rate will be obtained 15 and 30 minutes post procedure and recorded on the flowsheet.

4.2.5 Assessment of Imaged Lesions

At least two blinded radiologists will interpret the US images independently. Each interpreter will be asked to assess multiple lesion characteristics including echogenicity, size, septations, vascularity, and patterns of enhancement which will be used to classify the lesion. A diagnostic conclusion (e.g. benign, malignant or indeterminate) will be derived based on the Bosniak category if the lesion is cystic. Bosniak categories I, II and IIF will be classified as benign, and categories III and IV as malignant. The Bosniak classification system can be found in Appendix I. Readers will also be asked to score lesions based on a classification scheme presented by Barr in 2014.³⁰

Results of the perflutren-enhanced scans will not be used to dictate treatment and will not be shared with patients.

4.2.6 Truth Standard

Because the majority of patients will not undergo surgery/biopsy or receive a contrasted CT or MRI, the truth standard will be the change from baseline non-contrasted US at one year follow-up non-contrasted US, the most typical clinical approach to these lesions. Follow-up US will be categorized as either stable, regression or progression. Lesions that are stable or have regressed will be deemed a negative test. Lesions that have progressed will be positive. When clinically indicated, if tissue pathology/biopsy or other imaging studies including contrasted CT or MRI are available, these will be collected and used in secondary analyses.

4.3 Follow-up

For all patients, the treatment path will be decided by the subject's primary physician. We will review the medical records from each patient for a minimum of 12 months post CEUS and document results of 1-year urinalysis and serum creatinine and follow-up non-contrasted US 12 months after CEUS. Additionally, any

contrast-enhanced or non-contrast CT and/or MRI findings or surgical procedures performed as part of their routine standard of care will be collected and reviewed.

[REDACTED]
[REDACTED]

4.4 Duration of Study Intervention [REDACTED] [REDACTED]

The study intervention is complete once the patient receives perflutren-enhanced US (see Study Schema). We anticipate that CEUS imaging will last approximately 30 minutes for each subject, including set-up, pre-scan, and image capture. [REDACTED] The patient may be withdrawn from the study prior to this point if any of the following apply:

- Inter-current illness prevents completion of perflutren-enhanced US [REDACTED]
- Unacceptable adverse event(s) prevents completion of perflutren-enhanced US [REDACTED]
- Patient decides to withdraw from the study, **OR** [REDACTED]
- General or specific changes in the patient's condition render the [REDACTED] patient unacceptable for completion of perflutren-enhanced US in the judgment of the investigator.

4.5 Duration of Follow Up [REDACTED] [REDACTED]

Patients will be followed up via review of their medical records and through receipt of 1-year urinalysis and serum creatinine and a 1-year follow-up non-contrast US. Additional imaging studies and histopathological diagnoses will also be obtained for any scheduled biopsy and/or surgery as dictated by standard of care. Patients will also be followed indefinitely after the initial year for lesion changes/histologic diagnosis or changes in kidney function.

4.6 Removal of Patients from Protocol

Patients will be removed from study when any of the criteria listed in Section 4.4 apply. The Principal Investigator will be notified, and the reason for study removal and the date the patient was removed documented in the study documentation sheets. The patient should be followed-up per protocol.

5.0 DRUG INFORMATION [REDACTED] [REDACTED]

5.1 Perflutren Lipid Microspheres (Lantheus Medical Imaging) [REDACTED] [REDACTED]

The Definity® vial contains components that upon activation yield perflutren lipid microspheres composed of octafluoropropane. Perflutren is a diagnostic drug that is intended to be used for contrast enhancement. The vial contains a clear, colorless, sterile, non-pyrogenic, hypertonic solution which is activated by mechanical agitation with Vialmix®. [REDACTED]

Vialmix® is the activation device used in the preparation of US contrast imaging agents, including Definity®. Prior to activation, each Definity® vial contains 6.52 mg/mL octafluoropropane in the headspace and 0.75 mg lipid blend (0.045 mg DPPA, 0.401 mg DPPC, and 0.304 mg MPEG5000 DPPE), 103.5 mg propylene glycol, 126.2 mg glycerin, 2.34 mg sodium phosphate monobasic monohydrate, 2.16 mg

sodium phosphate dibasic heptahydrate and 4.87 mg sodium chloride in water in the clear liquid. Upon activation, each mL of the milky white suspension contains a maximum of 1.2×10^{10} perflutren lipid microspheres with approximately 150 $\mu\text{L/mL}$ octafluoropropane. [L] [SEP]

5.2 Supplier/How Supplied [L] [SEP]

Perflutren (Definity®) will be provided to study subjects at no cost. Perflutren is supplied as a single use 2mL clear glass vial containing clear liquid. Each package contains 4 single-use vials.

5.3 Handling and Dispensing of Perflutren [L] [SEP]

Perflutren lipid must be dispensed only from official study sites by authorized personnel according to local regulations. Perflutren should be stored in a secure area according to local regulations. It is the responsibility of the Investigator to ensure that study drug is only dispensed to study patients.

5.4 Storage Requirements/Stability [L] [SEP]

The drug product should be stored in a secure location with limited access under controlled temperature conditions of 2-8° C (36° -46° F) in a refrigerator. [L] [SEP]

5.5 Preparation [L] [SEP]

See <http://www.definityimaging.com/pdf/VIALMIX%20Users%20Guide.pdf>, and the package insert for instructions on the use of Vialmix®. [L] [SEP]

5.6 Dosage and Administration [L] [SEP]

See Section 4.2.4. [L] [SEP]

5.7 Clinical Safety Summary [L] [SEP]

See prescribing information on perflutren when used according to its FDA indication (<http://www.accessdata.fda.gov/scripts/cder/drugsatfda/> and highlights for perflutren prescribing at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021064s0111bl.pdf),

In addition, the following warnings and precautions are noted in the October 2011 labeling: [L] [SEP]

Serious Cardiopulmonary Reactions

Serious cardiopulmonary reactions including fatalities have occurred uncommonly during or shortly following perflutren-containing microsphere administration, typically within 30 minutes of administration. The risk for these reactions may be increased among patients with unstable cardiopulmonary conditions (acute myocardial infarction, acute coronary artery syndromes, worsening or unstable congestive heart failure, or serious ventricular arrhythmias). Always have cardiopulmonary resuscitation personnel and equipment readily available prior to

perflutren administration and monitor all patients for acute reactions. [L][SEP]The reported reactions include: fatal cardiac or respiratory arrest, shock, syncope, symptomatic arrhythmias (atrial fibrillation, tachycardia, bradycardia, supraventricular tachycardia, ventricular fibrillation, ventricular tachycardia), hypertension, hypotension, dyspnea, hypoxia, chest pain, respiratory distress, stridor, wheezing, loss of consciousness, and convulsions. [L][SEP]

Anaphylactoid Reactions [L][SEP]

In postmarketing use, uncommon but serious anaphylactoid reactions were observed during or shortly following perflutren-containing microsphere administration including: shock, hypersensitivity, bronchospasm, throat tightness, angioedema, edema (pharyngeal, palatal, mouth, peripheral, localized), swelling (face, eye, lip, tongue, upper airway), facial hypoesthesia, rash, urticaria, pruritus, flushing, and erythema. These have occurred in patients with no prior exposure to perflutren-containing microsphere products. [L][SEP]

Systemic Embolization of Perflutren in Patients with Cardiac Shunts [L][SEP]

In patients with right-to-left, bi-directional, or transient right-to-left cardiac shunts phospholipid-encapsulated microspheres can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation resulting in microvascular occlusion and ischemia. In an animal study utilizing intra-arterial administration of activated perflutren, microsphere trapping was seen in small arterioles <15 µm, especially at branch points and in capillaries at all doses tested, including doses directly applicable to those used in humans. An animal study utilizing intravenous [L][SEP]administration did not result in arterial microvascular obstruction presumably because of filtering by the lungs. Do not administer perflutren by intra-arterial injection.

High Ultrasound Mechanical Index [L][SEP]

High ultrasound mechanical index values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. Additionally, end-systolic triggering with high mechanical indices has been reported to cause ventricular arrhythmias. The safety of activated perflutren at mechanical indices greater than 0.8 has not been evaluated.

QTc Prolongation [L][SEP]

ECG parameters for doses up to 10 µL/kg were monitored in 221 subjects at multiple time points from 1 hour to 72 hours after the first bolus injection. In the 221 subjects, QTc prolongations of >30 msec were noted in 64 (29%) subjects. Forty-six out of 64 subjects with QTc prolongations were further evaluated and 39% (18/46) showed associated cardiac rhythm changes. The effects of concomitant drugs were not studied.

5.8 Return and Retention of Study Drug

Remaining reagent at the completion of the study or expired reagent will be destroyed.

6.0 EVALUATIONS AND ASSESSMENTS

[L]
[SEP]

6.1 Pre-Study Assessments [L] [SEP]

Written informed consent will be obtained from the subjects prior to the implementation of any study procedures. The subject will receive a signed and dated informed consent form. A complete medical history will be taken at enrollment and reviewed on presentation for the study ultrasound. A serum or urine β -HCG test will be performed within 24 hours prior to perflutren administration if clinically appropriate. [L]
[SEP] All medications (prescription and over-the-counter) taken within 24 hours prior to perflutren lipid administration will be recorded.

[L]
[SEP]

6.2 Treatment Assessments [L] [SEP]

Subjects will be monitored for any untoward medical occurrences or toxicities from the time of signed informed consent through 30 minutes after perflutren lipid administration. [L]
[SEP]

6.3 Follow-up Assessments [L] [SEP]

All post-CEUS clinical diagnoses based on 1-year follow-up non-contrasted US will be reviewed. Any post-operative histopathological diagnoses for patients who undergo biopsy/surgery within the 12-month follow-up period post perflutren lipid administration will be reviewed. Any additional non-contrasted or contrasted imaging (CT or MRI) within the 12-month follow-up period post perflutren lipid administration will be reviewed.

6.4 Assessment of Safety [L] [SEP]

Any patient in whom perflutren administration is initiated will be evaluable for toxicity.

Baseline creatinine (within 12 weeks prior to study) and urinalysis (on day of study, pre and post-CEUS) will be collected from each patient prior to CEUS. At and 1-year post-CEUS, serum creatinine levels and urinalysis will be obtained from primary providers. Serum creatinine from a comparison group drawn from patients who meet study inclusion and exclusion criteria but decline to participate in the study will be followed over the study period to detect potential adverse effects the study intervention may have on kidney function of the study subjects. Their serum creatinine values will be collected at 1-year post initial recruitment with a minimum of 1 value at 1-year timepoint.

6.5 Tissue Collection and Optional Storage [L] [SEP]

If a patient undergoes nephrectomy, all nephrectomy specimens will be collected by

the tissue procurement facility. Final diagnoses will be obtained by chart review. [L][SEP]

7.0 ADVERSE EVENTS

7.1 Definitions [L][SEP]

7.1.1 Adverse Event (AE) [L][SEP]

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product. Each AE will be graded on a mild-moderate-severe scale.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

7.1.2 Suspected Adverse Reaction (SAR) [L][SEP]

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. Each SAR will be graded on a mild-moderate-severe scale.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome) [L][SEP]
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than one occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event. [L][SEP]
- An aggregate analysis of specific events observed in a clinical trial that

indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

7.1.3 Unexpected AE or SAR ^[1]_{SEP}

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB 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.

7.1.4 Serious AE or SAR ^[1]_{SEP}

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes: ^[1]_{SEP}

- Death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- 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 the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

7.2 Documentation of non-serious AEs or SARs ^[1]_{SEP}

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through 24 hours after treatment is discontinued.

^[1]_{SEP} Collected information should be recorded in the Case Report Forms (CRF) for that patient. A description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug will be documented.

7.3 SAEs or Serious SARs

7.3.1 Timing ^[L]_[SEP]

For any experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued.

7.3.2 Documentation and Notification ^[L]_[SEP]

These events (SAEs or Serious SARs) must be recorded within 24 hours of learning of its occurrence.

7.3.3 Reporting ^[L]_[SEP]

IRB Reporting Requirements:

- UNC will submit an aggregated list of all SAEs to the UNC IRB annually at the time of study renewal according to the UNC IRB policies and procedures. ^[L]_[SEP]
- The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system (see section 9.4.3) within 7 days of the Investigator becoming aware of the problem.

Pregnancy ^[L]_[SEP]

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study should be recorded as SAEs. The patient is to be discontinued immediately from the study. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must document the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

FDA Expedited Reporting requirements for studies conducted under an IND:

If an investigator deems that an event is both a serious SAR AND unexpected, it must also be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. The MedWatch 3500a form can be accessed at:

<http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm>. (Please be sure and access form 3500a, and not form 3500). For Affiliate Investigators, the MedWatch form should be faxed to the UNC Study Coordinator at 919-966-0817 along with supporting documentation defining the event and

causality. UNC, as the Sponsor of the study, will make the final determination regarding FDA submission.

Once the UNC Principal Investigator determines an event is a serious SAR AND unexpected, the MedWatch 3500A form will be submitted to the FDA.

The UNC Study Coordinator will also be responsible for informing each Affiliate site of all serious and unexpected SARs reported to the FDA via fax as soon as possible.

7.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data including the number of patients treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

7.4.1 Monitoring Safety of Participants

Blood and urine test results within 12 weeks of study recruitment will be obtained from the subject's clinical provider at the time of enrollment, as a part of the standard of care disease management and for determination of inclusion (unless the patient has a transplant or is on dialysis). In particular, serum creatinine and any

evidence of hematuria will be noted. Urinalyses will be obtained just prior and post-CEUS to detect any evidence of hematuria. Serum creatinine measurements and urinalyses will be followed during the duration of study follow-up with intervals at the discretion of the primary provider. This information will be compiled by study coordinators by review of the medical chart and when necessary, obtaining records directly from the clinical provider.

7.4.2 Individual Subject Stopping

Patient safety will be considered during the study intervention with individual patient withdrawal for criteria outlined in 4.4 and managed as described in 4.6. There are no subsequent interventions after the initial contrast-enhanced ultrasound.

7.4.3 Study-wide Stopping

If there is a severe SAR or a severe AE suspected to be related to the study intervention, CEUS, the study will be put on hold and re-evaluated as this would not be an expected event.

7.4.4 Ensuring data accuracy and protocol compliance

The PI will monitor the accruing data and make decisions about the ongoing safety of the trial. A Medical Monitor outside the study will be asked to provide an independent review of the study and data. The PI will be responsible for providing the following information for such a review: 1) safety and accrual data including the number of patients treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary diagnostic data; and 4) summaries of team meetings that have occurred since the last report.

8.0 STATISTICAL CONSIDERATIONS ^[L]_[SEP]

8.1 Study Design/Study Endpoints ^[L]_[SEP]

This is an investigator-initiated, prospective pilot study evaluating the sensitivity of contrast enhanced ultrasound using microbubble contrast agent (perflutren lipid; Definity®) in diagnosing renal malignancy in patients with kidney disease and an indeterminate or possibly malignant kidney mass on a screening CT/MR/US. The study is designed to estimate the sensitivity of CEUS as a surrogate for conventional imaging.

8.2 Sample Size and Accrual ^[L]_[SEP]

This study will recruit to a target goal of 20 patients total which will be combined with 75 patients from a previous study (15-1866) under a similar protocol. Both studies use the same inclusion/exclusion criteria and patient population. However, this study has slightly modified imaging techniques based on the information learned from the 15-1866.

We will also include 5 healthy volunteers for image optimization, as described on page 13.

We expect to complete the enrollment in 6 months. Based on available literature regarding incidence of malignancy in indeterminate (Bosniak IIF and III) lesions^{31, 32} and accuracy of CEUS for cystic kidney lesion characterization in a general, non-CKD population^{9, 16, 22, 30, 33} and our experience from a pilot study including 27 patients with kidney disease and an indeterminate lesion, we approximate a true positive rate of 10%, false positive rate of 8%, true negative rate of 80% and false negative rate of 2%. Given the sample size of 75 (from previous study) and these approximated rates, we project an achievable sensitivity of 83%, specificity of 91%, PPV of 56% and NPV of 98%. The 95% confidence interval for this sensitivity estimate is 74.5-91.5% with a width of 17%. With the additional 20 lesions, we can improve confidence in our sensitivity and specificity measures.

8.3 Data Analysis Plans

Primary analyses will include 1) estimating the sensitivity of CEUS qualitative interpretations using the truth standard of findings on 1-yr follow-up non-contrasted US compared to baseline non-contrasted US and 2) comparing the sensitivity and specificity of two different classification schemes for classifying lesions on CEUS images compared to the truth standard of findings on 1-yr follow-up non-contrasted US compared to baseline non-contrasted US.

Descriptive summary statistics such as mean, median and standard deviation of tumor measurement will be provided to quantify and characterize kidney lesions. Similarly, other patient clinical and demographic characteristics will be summarized, using frequencies in the case of categorical variables.

For the primary objective, sensitivity and specificity and their 95% confidence intervals will be computed for CEUS qualitative interpretations compared to change in baseline non-contrasted US. Based on the truth standard diagnosis of lesion progression at one year follow-up, a 95% confidence interval for the Mantel-Haenszel odds ratio will be constructed to quantify the odds that a subject with a diagnosis of a malignant lesion by non-contrasted US will be diagnosed with a malignant lesion by CEUS relative to the odds for a subject without a malignant lesion diagnosis by non-contrasted US. A similar odds ratio and 95% confidence interval will be constructed for subjects with a truth standard diagnosis of stable or lesion regression. Sensitivities, specificities are based on diagnoses made using CEUS-based risk classification (class I, II, and IIF considered negative, class III and IV as positive, using modified Bosniak criteria). Estimation of sensitivity and specificity will be carried out for each reader. Agreement between readers will be based on kappa statistics for the dichotomous diagnosis and using weighted kappas based on the ordinal Bosniak scale.

As part of a the secondary object, a sensitivity analysis, the sensitivity of CEUS (qualitative interpretation metrics), will be compared to the sensitivity when other standards are incorporated when available, including pathology and additional imaging with contrasted or non-contrasted CT or MR.

For the secondary objective, the prediction performance of the two different classification schemes will be compared by generating sensitivity, specificity and their 95% confidence intervals compared to the truth standard.

9.0 STUDY MANAGEMENT

9.1 Institutional Review Board (IRB) Approval and Consent ^[L]_[SEP]

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) Good Laboratory Practice (GLP) and to ethical principles that have their origin in the Declaration of Helsinki. Before recruitment and enrollment into this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form. ^[L]_[SEP] Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

9.2 Required Documentation ^[L]_[SEP]

Before the study can be initiated at any site, the following documentation must be provided to the Study Sponsor, Lantheus.

- A copy of the official IRB approval letter for the protocol and informed consent ^[L]_[SEP]
- CVs and medical licensure for the principal investigator and any co-investigators who will be involved in the study
- Form FDA 1572 appropriately filled out and signed with appropriate ^[L]_[SEP] documentation (NOTE: this is required if UNC holds the IND. Otherwise, the Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol is sufficient to ensure compliance) ^[L]_[SEP]
- Executed clinical research contract

^[L]_[SEP]

9.3 Data Management and Monitoring/Auditing ^[L]_[SEP]

The Radiology Research Department will serve as the coordinating center for this trial. Data will be collected on paper charts, translated into Excel flowsheets and housed in the Radiology Research Department. Radiology Research Department will coordinate and manage data for quality control assurance and integrity. There will be three components of data collection: 1) CEUS data, stored in the Dayton lab under the unique identifier; 2) general patient data, stored in the radiology study coordinator database; 3) study specific patient clinical information including blood and urine tests and “truth standard” conventional US results and any additional imaging tests or histology, stored in the study coordinator and PI’s office on a password-protected, secured network drive.

All data will be collected and stored by Study Coordinators from the Radiology Research Department. All electronic data will be stored on a password-protected, secured computer. The study files will be password-protected. Paper records will be housed in a secured lockbox in a locked office. The investigators will allow monitors to review all source documents supporting stored data.

The data management plan includes assigning a unique identifier to each patient. For example, 101, 102, 103, etc. If a patient has multiple lesions, these lesions will be designated A, B, etc. The numbering will begin where IRB study 15-1866 left off. These will be the unique de-identified codes for each lesion with the master code held in the radiology study coordinator’s office in a separate, password-protected database. Prior to statistical analysis, a general quality control will be performed to look for invalid entries/out of range findings/outliers. Only principal investigators, research coordinators and members of Dr. Dayton’s lab performing image and data analysis will have access to study data. The study biostatistician will only have access to de-identified data (with the possible exception of dates that are clinically meaningful, e.g., study visit dates).

^[L]_[SEP]**9.4 Adherence to the Protocol** ^[L]_[SEP]

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

9.4.1 Emergency Modifications ^[L]_[SEP]

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favorable opinion. For any such emergency modification implemented, a UNC IRB modification form must be completed by Research Personnel within five (5) business days of making the change.

9.4.2 Single Patient/Subject Exceptions ^[L]_[SEP]

Any request to enroll a single subject who does not meet all the eligibility criteria of

this study requires the approval of the Principal Investigator and the IRB.

9.4.3 Other Protocol Deviations/Violations [L] [SEP]

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that: [L] [SEP]

- Is generally noted or recognized after it occurs [L] [SEP]
- Has no substantive effect on the risks to research participants [L] [SEP]
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected [L] [SEP]
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria: [L] [SEP]

- Has harmed or increased the risk of harm to one or more research participants [L] [SEP]
- Has damaged the scientific integrity of the data collected for the study
- Results from willful or knowing misconduct on the part of the [L] [SEP] investigator(s) [L] [SEP]
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies. [L] [SEP]

If a deviation or violation occurs please follow the guidelines below: [L] [SEP]

Protocol Deviations: UNC Research personnel will report the deviation to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review. [L] [SEP]

Protocol Violations: Violations should be reported within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems. [L] [SEP]

Unanticipated Problems: [L] [SEP] Any events that meet the criteria for "Unanticipated Problems" as defined by the IRB must be reported by the Study Coordinator using the IRB's web- based reporting system.

9.5 Amendments to the Protocol [L] [SEP]

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required. The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

9.6 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

9.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/GLP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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CEUS CKDx

PI: Emily Chang

April 2, 2018

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11.0 APPENDICES**Appendix I**

The Bosniak classification system is as follows³⁴:

TABLE I. Bosniak renal cyst classification system

Category	Description
I	A benign simple cyst with a hairline thin wall that does not contain septa, calcifications, or solid components. It measures water density and does not enhance.
II	A benign cyst that may contain a few hairline thin septa in which "perceived"* enhancement may be present. Fine calcification or a short segment of slightly thickened calcification may be present in the wall or septa. Uniformly high attenuation lesions <3 cm (so-called high-density cysts) that are well marginated and do not enhance are included in this group. Cysts in this category do not require further evaluation.
IIF (F for follow-up)	Cysts that may contain multiple hairline thin septa or minimal smooth thickening of their wall or septa. Perceived enhancement of their septa or wall may be present. Their wall or septa may contain calcification that may be thick and nodular, but no measurable contrast enhancement is present. These lesions are generally well marginated. Totally intrarenal nonenhancing high-attenuation renal lesions >3 cm are also included in this category. These lesions require follow-up studies to prove benignity.
III	"Indeterminate" cystic masses that have thickened irregular or smooth walls or septa in which measurable enhancement is present. These are surgical lesions, although some will prove to be benign (eg, hemorrhagic cysts, chronic infected cysts, and multiloculated cystic nephroma), some will be malignant, such as cystic renal cell carcinoma and multiloculated cystic renal cell carcinoma.
IV	These are clearly malignant cystic masses that can have all the criteria of category III, but also contain enhancing soft-tissue components adjacent to, but independent of, the wall or septum. These lesions include cystic carcinomas and require surgical removal.

* Not measurable enhancement—see text for details.

Appendix 2**Time and Events Table**

	Screening	Pre-CEUS Visit	CEUS study	Mo12 (Wk 48)	Early Termination ¹
Inclusion/Exclusion Criteria	X				
Comprehensive Medical History	X ²	X ²			
Informed Consent	X ³	X ³			
Education of potential side effects		X			
Pregnancy test (urine), if applicable		X ⁴			
Serum creatinine	X			X ⁵	X ⁵
Urinalysis		X ⁶		X ⁶	
Contrast-Enhanced US (CEUS)			X ⁷		
AE Assessment			X ⁸		
Request tissue diagnosis				X ⁹	
Obtain follow-up conventional US and any other imaging studies				X	X

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PI: Emily Chang

April 2, 2018

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obtained during the follow-up period					
Patients declining CEUS but allowing creatinine follow-up¹⁰					
Serum creatinine	X			X	X

¹For any patients that do not complete the CEUS study, an ongoing adverse events/serious adverse events will be followed to the resolution of the event. For any patient that is deceased before the 1-year follow-up most recent creatinine levels and imaging studies will be collected and autopsy results documented, if available.

²Comprehensive medical history obtained at time of enrollment; thereafter history focused on symptoms and assessments.

³Consent can be obtained and signed at either screening visit or during the pre-study visit. It will always be reviewed at pre-study visit, even if signed during screening visit.

⁴Women of childbearing potential must have negative urine or serum pregnancy test on the day of CEUS study, prior to the study.

⁵Serum creatinine levels will be obtained clinically at the discretion of the patient's primary provider. We will obtain results of serum creatinine levels drawn by the primary provider.

⁶Urinalysis will be obtained prior to and post CEUS study. This will be obtained by Radiology Nursing staff as per agreement. Results will be obtained and recorded by Study Coordinator. At approximate 6 and 12-month intervals, urinalysis will also be obtained. This can be done locally with results sent to UNC.

⁷During the CEUS study, Study Coordinator will record time of injection.

⁸Adverse event assessment will happen during the CEUS study and the 30-minute post-study monitoring period, where they will be monitored by the Radiology nursing staff with vital signs (blood pressure and heart rate) recorded in the hospital's electronic monitoring system. These results will be collected by the Study Coordinator and documented in the Monitoring Logs/Flowsheets housed in the Radiology Research Coordinator Office.

⁹For patients who undergo biopsy or nephrectomy, tissue diagnosis will be requested after the procedure.

CEUS CKDx

PI: Emily Chang

April 2, 2018

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¹⁰For patients who decline study enrollment with CEUS but meet all study inclusion/exclusion criteria but agree to allow us to collect creatinine values from their primary providers over the study period, serum creatinine will be collected, if available at the designated time periods with a minimum value at 1-year.