CROSS-SECTIONAL STUDY OF CONTRAST-ENHANCED ULTRASOUND WITH LUMASON/DEFINITY AS A SCREENING TOOL FOR KIDNEY CANCER IN PATIENTS WITH VON-HIPPEL LINDAU

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Study Product: *Perflutren Lipid Microsphere (Definity)*
Sulfur Hexafluoride Lipid (Lumason)

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List of Abbreviations

VHL – von Hippel-Lindau
CEUS – Contrast-enhanced ultrasound
US – ultrasound
CT – computed tomography
MRI – magnetic resonance imaging
RCC – renal cell carcinoma
NSF – nephrogenic sclerosing fibrosis
FDA – Food and Drug Administration
CPS – cadence pulse sequencing
BHD – Birt-Hogg-Dube syndrome
HLRCC – Hereditary Leiomyomatosis and Renal Cell Carcinoma
TSC – Tuberous Sclerosis Complex (TSC).

Study Summary

Title	Cross-Sectional Study of Contrast-Enhanced Ultrasound with Lumason/Definity as a Screening Tool for Kidney Cancer in Patients with Von Hippel-Lindau
Short Title	CEUS VHL
Protocol Number	LCCC1824
Phase	NA
Methodology	Cross-sectional Observational Study
Study Duration	12 months
Study Center(s)	UNC-CH
Objectives	To determine accuracy of CEUS for detection of malignancy kidney lesions in patients with Von-Hippel Lindau
Number of Subjects	15
Diagnosis and Main Inclusion Criteria	Von-Hippel Lindau, Birt-Hogg-Dube syndrome, Hereditary Leiomyomatosis, Renal Cell Carcinoma, or Tuberous Sclerosis Complex Key inclusion criteria: Undergoing kidney surveillance with MRI
Study Product, Dose, Route, Regimen	Microbubble contrast agent (IV infusion): 1. Perflutren Lipid Microspheres (Definity) 2. Sulfur hexafluoride Lipid (Lumason)
Duration of administration	15 minutes
Reference therapy	Contrast-enhanced MRI
Statistical Methodology	Kappa statistics

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Von-Hippel Lindau (VHL) is one of several inherited familial syndromes that involves multiple organs and predisposes patients to the development of various benign and malignant tumors in these organs, including the central nervous system (CNS), kidney, pancreas, and adrenal glands. Renal Cell Carcinoma (RCC) is the leading cause of death in patients with VHL. Approximately two-thirds of patients with VHL will develop kidney cysts, and the frequency of RCC varies from 25-70% depending on age[1]. The Bosniak criteria, a classification system used to classify kidney cysts, is used to define the cystic lesions present in VHL. The Bosniak criteria are typically applied to contrast-enhanced CT or MRI. Consequently, surveillance guidelines recommend screening with yearly ultrasound and every other year contrast-enhanced MRI[2]. Other familial syndromes at risk of kidney cancer that require routine lifelong imaging surveillance include Birt-Hogg-Dube syndrome (BHD), Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC) and Tuberous Sclerosis Complex (TSC).

The mean age of diagnosis of RCC in VHL is 40 years[3], but atypical presentations have occurred in patients as young as 16[4]. Therefore, VHL Alliance Screening Guidelines recommend beginning active surveillance with yearly ultrasound and every other year contrast-enhanced MRI at age 16[2]. In addition to abdominal imaging, CNS MRI using contrast is recommended every 2-3 years[2]. Therefore, patients that live to their 70s may be exposed to >50 contrast-enhanced MRI scans in a lifetime. Routine imaging surveillance is also a major component of management in BHD, HLRCC and TSC at variable screening intervals.

Gadolinium was once considered a very safe contrast agent for MRI with a low adverse event rate and much lower rates of allergic response compared to iodinated contrast used with CT. In 2006 a disease called Nephrogenic Sclerosing Fibrosis (NSF), a devastating disease leading to fibrosis of various organs, was described and found to be highly correlated with the use of gadolinium in patients with impaired kidney function[5,6]. Changes in prescribing practices led to the elimination of NSF, but a new entity, Gadolinium Deposition Disease, is now described in which dose-related gadolinium deposition was found in the brains of autopsy patients receiving gadolinium but not controls[7,8]. Although the clinical significance of this is not yet clear, avoidance of multiple repeated doses of gadolinium and costly MRI tests may benefit any patient, but particularly those requiring large lifetime doses.

Contrast-enhanced ultrasound (CEUS) is an emerging imaging technology that is being investigated for use in characterizing indeterminate kidney lesions[9]. Thus far, results have shown excellent sensitivity and moderate specificity, both comparable to contrast-enhanced CT and MRI. Microbubbles, the contrast agent used in CEUS have thus far proven to be quite safe based on decades of clinical use in cardiac imaging[10]. The rate of allergic reaction is similar to gadolinium, and microbubbles are not nephrotoxic. For certain patients, replacement of contrast-enhanced MRI with CEUS may significantly reduce lifetime exposure to gadolinium as well as costs associated with an expensive test.

1.2 Investigational Agent

Contrast-Enhanced Ultrasound Diagnostic Imaging of the Kidney

Ultrasound (US) imaging of the kidney is typically performed in the United States without contrast as no US contrast agent is currently approved for this indication. Without contrast, malignant tumors often cannot be definitively differentiated from benign tumors or pseudotumors. For this reason, following the identification of a kidney lesion via US without contrast, contrast-enhanced CT or MRI is then performed to stage and grade the lesion.

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, remain

intravascular for several minutes, and do not diffuse into the interstitium (pure blood-pool agents)[11]. Three US contrast agents are Food and Drug Administration (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. The intent of this study is not to change the labeling of either FDA-approved agent.

Contrast Agents

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 setting, the maximum dose of perflutren is administered as either bolus doses or one single intravenous infusion.

Lumason® (sulfur hexafluoride lipid) is an FDA-approved US contrast agent indicated for use in adult patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border and with ultrasound of the liver in adult and pediatric patients to characterize focal liver lesions. See http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/203684s001lbl.pdf for full prescribing information.

Associated Toxicities

Most safety studies have been performed in cardiac patients as this was the only FDA approved indication until April 4, 2016 when Lumason® was approved for liver applications. Thus far, microbubble agents have been shown to be quite safe[12,13]. 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, 1 due to a hypersensitivity reaction and the rest due to dizziness, chest pain, dyspnea or back pain. The events appeared within 1 – 15 minutes of the drug administration and were of moderate intensity resolving usually without treatment within minutes or hours after onset. 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. 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.

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%).

Initial post-marketing experience, which included over 1 million patients with 5 years surveillance, showed the only medically significant risk was rare allergic events, occurring at a rate of 1 in 10,000[13].

Cardiopulmonary Reactions

Cardiopulmonary contraindications include cardiac shunts and hypersensitivity to perflutren. The initial mandated 30-minute monitoring period was limited to patients with pulmonary hypertension or unstable cardiopulmonary conditions. The mandatory 30-minute monitoring period was removed in 2011, although a statement remained 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.

Although recent studies have shown no increased risk in patients with pulmonary hypertension[14], patients with a history of known cardiac shunts, pulmonary hypertension or unstable cardiopulmonary conditions

will be excluded from our study. In addition, any patient with a suspected reaction during imaging will be monitored 30-minutes post-contrast agent administration by clinical or research nurse.

Hypersensitivity Reactions
Anaphylaxis to perflutren is extremely rare, however, in case of a severe reaction, EpiPen® (epinephrine) injections will be kept near the US machine for all patients.

High Ultrasound Mechanical Index (MI)
While high ultrasound MI of the heart may cause microbubble cavitation and subsequent ventricular arrhythmias, this was seen only at MI>1.6, higher than the typical MI used for microbubble disruption, with an agent no longer in clinical use.[15] No premature ventricular contractions were seen with MI≤1.1. Large clinical trials using MI≤1.0 for expanded cardiac indications have shown no significant concerns for arrhythmias[13,16,17].[13,16,17]

Nevertheless, the safety of perflutren at MIs >0.8 has not yet been established by the manufacturer. However, real-time measurement of renal blood flow in 19 healthy subjects using perflutren was 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 20mmHg 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[18]. A second study using 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[19].

Small pre-clinical studies performed in rodents and pigs[20-23] showed conflicting results. One group showed glomerular capillary hemorrhage in exposed animals while other groups showed no sign of damage. The clinical relevance of these findings has not yet been investigated and is believed to be minimal, if any.

1.3 Preclinical Data

Numerous pre-clinical studies of CEUS have been performed, primarily in rodent kidneys but also larger animals like rabbit and pig, that investigate overall perfusion of diseased kidneys[24-26] or kidneys exposed to various medications[27,28]. In the field of oncology, many pre-clinical studies have been performed to determine the sensitivity of CEUS for detection of malignancy[29-31]. The use of targeted microbubbles (with antibodies attached to the microbubble surface) has been investigated in this field as well[32,33].

1.4 Clinical Data to Date

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-time[34-40]. Outside of the United States, or in the context of clinical trials, US contrast agents have been used in kidney imaging[36,37,40-44]. 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 kidney pseudotumors[45], cystic kidney lesions[37,41,43,46], and solid kidney lesions[40,44].

In the United States, where there has been significantly less research on using US contrast in humans, the US contrast agent, Definity®, was used in one study to measure renal blood flow in a healthy population[18], effectively reproducing previous animal studies in the human population. Multiple contrast agents were used in a large retrospective study of CEUS in patients with kidney lesions[47] and showed excellent sensitivity (100%) and specificity (99%). Since that study, several smaller studies in the United States have looked at CEUS in the kidney and consistently shown excellent sensitivity[48,49]. Studies outside the United States show similar sensitivities and specificities[9,50].

1.5 Dose Rationale and Risk/Benefits

Dosages for both Definity and Lumason are based on package inserts, expert opinion from radiologists who routinely use CEUS for kidney imaging, and our previous experience from clinical trials (IRB 12-2314, 15-1866, 17-1130). Because the kidney is a very vascular organ, use of 0.2mL Definity has been adequate for visualization of lesion enhancement. Our experience has been with both bolus doses of 0.2mL and infusion at set rates based on BMI. The infusion rate can be increased or decreased if visualization is not adequate. Use of either small boluses or infusion allows for multiple injections for patients with multiple lesions. Patients with greater abdominal girth and multiple lesions may require a 2nd dose as their bolus doses or rate of infusion will be higher. In the case of a 2nd dose, a 30-minute interval between injections will be instituted. All microbubble contrast agents are injected intravenously.

The greatest risk is of an unknown hypersensitivity reaction to the microbubbles as many patients will not have previous exposure to these contrast agents. However, the rate of true allergic reactions is low and similar to that for gadolinium-based contrast agents. Other risks are transient and resolve when the contrast agent is cleared. Overall the risk is minimal. Benefits include improved visualization of kidney lesions compared to non-contrasted ultrasound and the potential reduction of total lifetime load of gadolinium and improvement in cost-effectiveness.

2 Study Objectives

Primary Objective
To assess the agreement between CEUS and routine B-mode US for detecting indeterminate features of kidney lesions as graded on a Bosniak scale in patients with VHL under abdominal imaging surveillance.

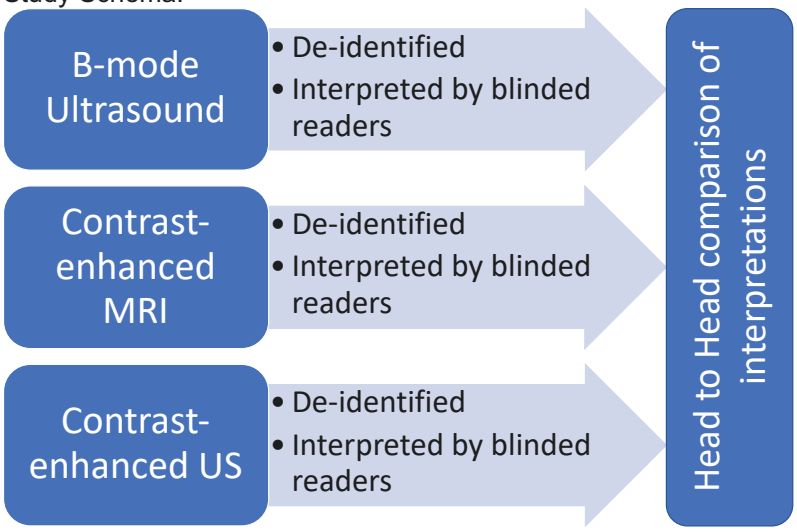
Secondary Objective
To assess the agreement between CEUS and contrast-enhanced MRI for detecting indeterminate features of kidney lesions as graded on a Bosniak scale in patients with VHL under abdominal imaging surveillance.

3 Study Design

3.1 General Design

This study is a pilot cross-sectional study.

Study Schema:



Any patient with a familial syndrome at risk of kidney cancer (VHL, BHD, HLRCC or TSC) undergoing annual imaging screening is eligible, but we will target inclusion of at least 10 subjects who have at least 1 kidney lesion. Therefore, up to 5 subjects may have no current kidney lesions. Subject participation will be

only for the day of CEUS study. There will be no follow-up period for this study. However, if results are encouraging, a longitudinal observational study may follow, and these same subjects would be eligible for enrollment.

Abdominal imaging in patients with VHL is also used to monitor the pancreas. Although pancreatic lesions are less common than kidney lesions, cross-sectional imaging allows for monitoring of both organs. For patients with VHL, we will image the pancreas in addition the kidneys in order to establish parameters for pancreas CEUS. CEUS of the pancreas is not novel[51], but its use for screening in patients at risk of pancreas lesions has not been investigated.

Following completion of imaging, all CEUS, MRI (within 4 months) and B-mode (at time of CEUS) US studies will be de-identified. Blinded radiologists will interpret images and provide an overall assessment of risk of malignancy to each kidney using the Bosniak criteria for each kidney lesion present. The Bosniak criteria places cystic lesions into one of 5 categories (I, II, IIF, III and IV) based on lesion characteristics. CEUS based diagnosis will be compared to the diagnoses on routine B-mode US and contrast-enhanced MRI.

3.2 Primary Study Endpoints

The primary study endpoint will be agreement of qualitative radiologist review of CEUS images with routine B-mode US.

3.3 Secondary Study Endpoints

The secondary study endpoint will be agreement of qualitative radiologist review of CEUS images with contrast-enhanced MRI.

3.4 Primary Safety Endpoints

- Adverse effects of microbubble contrast administration.
- Development of hematuria post-CEUS.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

To be eligible for the present study, patients must meet the following criteria:

4.1.1 Able to provide written informed consent

4.1.2 Willing to comply with protocol requirements

4.1.3 At least 18 years of age

4.1.4 Carry a diagnosis of VHL, BHD, HLRCC or TSC and able to undergo routine clinical screening tests for RCC

4.2 Exclusion Criteria

4.2.1 Critically ill or medically unstable or in an intensive care setting and whose critical course during the observation period would be unpredictable

4.2.2 Known hypersensitivity to sulfur hexafluoride or to any component of perflutren lipid (Definity®) or sulfur hexafluoride (Lumason®)

4.2.3 Right to left shunt, severe pulmonary hypertension (Pulmonary artery pressure >90mmHg), or adult respiratory distress syndrome

4.2.4 Active cardiac disease including any of the following:

- Severe congestive heart failure (class IV in accordance with the classification of the New York Heart Association)
- Unstable angina.
- Symptomatic arrhythmia (i.e. tachycardia, bradycardia, supraventricular tachycardia, ventricular fibrillation, ventricular tachycardia, atrial flutter or fibrillation).
- Myocardial infarction within 14 days prior to the date of proposed microbubble administration.

4.2.5 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:

- Mental illness
- Drug abuse

4.2.6 Female patient who is pregnant or lactating

4.2.7 Obesity that limits obtainment of acceptable images

4.3 Subject Recruitment and Screening

Subjects will be recruited from the UNC von Hippel-Lindau Clinical Care Center, under the directorship of Mary Dunn, NP and Dr. Tracy Rose. UNC currently has 20-25 patients who undergo annual screening. No specific handouts will be used as the target population is very specific. Principal investigator and sub-investigators will approach patients that meet inclusion and exclusion criteria to determine interest in participation. There are no laboratory tests required. Prior imaging to determine if any lesions are present (as we are targeting at least 10 subjects who have at least one lesion) will be required.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

The patient may be withdrawn from the study prior to this point if any of the following apply:

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

Subject will be monitored for a minimum of 30 minutes following any possible adverse event related to contrast agent as most reported adverse reactions resolved with clearance of the contrast agent. The Principal Investigator will be notified, and the reason for study removal documented in study documentation sheet.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Any subject who is withdrawn from the study will be followed in the immediate post-imaging period and for 72 hours following contrast administration, if contrast agent was administered, for the purpose of detecting any unanticipated adverse effect of contrast administration. If no contrast agent was administered, follow-up will not be required. Since this is a cross-sectional study, and subjects are not being followed longitudinally, subjects that withdraw will also not be followed beyond the 72-hour post-imaging period if complete resolution of any adverse events is noted at 72 hours. If adverse effects are persistent, subjects will continue to be followed until resolution of adverse effects.

5 Study Drug

5.1 Description

5.1.1 Perflutren Lipid Microspheres (Lantheus Medical Imaging)

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®.

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 µL/mL octafluoropropane.

5.1.2 Sulfur hexafluoride lipid microspheres (Bracco Diagnostics Inc)
If Definity is not available, Lumason will be used as a secondary contrast agent.
Lumason (sulfur hexafluoride lipid-type A microspheres) comes in single-patient use kits whic contain the following three items:
1) one clear glass 10 mL vial containing 25 mg of lyophilized powder lipid-type A, 60.7 mg of sulfur hexafluoride gas and capped with a blue flip-cap
2) one prefilled syringe containing 5 mL Sodium Chloride 0.9% Injection, USP (Diluent)
3) one Mini-Spike

Each vial is formulated as a 25 mg sterile, pyrogen-free lyophilized powder containing 24.56 mg of polyethylene glycol 4000, 0.19 mg of distearoylphosphatidyl-choline (DSPC), 0.19 mg of dipalmitoylphosphatidylglycerol sodium (DPPG-Na) and 0.04 mg of palmitic acid. The headspace of each vial contains 6.07 mg/mL (± 2 %) sulfur hexafluoride, SF6, or 60.7 mg per vial.

Each prefilled syringe with 5 mL of diluent 0.9% Sodium Chloride Injection is sterile, nonpyrogenic, preservative free containing 9 mg sodium chloride per mL. Upon reconstitution with 5mL diluent, Lumason is a milky white, homogeneous suspension containing sulfur hexafluoride lipid-type A microspheres.

5.2 Contrast Administration

The contrast agent will be dosed undiluted (0.2-0.3 mL Definity) for up to 7 intravenous injections, depending on the number of lesions (1 injection per kidney and 1 for pancreas in VHL patients, and 1 injection per lesion with remaining injections available per vial if wash-in of the contrast was not captured). The activated undiluted dose of 0.2-0.3 mL will be given as a bolus injection, followed by a total of 5 mL saline flush after the contrast agent is fully injected.

5.3 Method for Assigning Subjects to Treatment Groups

Subjects are not randomized for this study. They will be selected based on prior imaging and meeting the inclusion criteria.

5.4 Preparation and Administration of Study Drug

The contrast agent, Definity (or Lumason), will be dispensed from the UNC Investigational Drug Service (IDS). It will be dispensed in inactivated form to a study team member. The contrast agent will be activated just prior to administration (ideally to be used within 5 minutes of activation). Contrast agent will be activated by nursing staff trained in the activation of Definity (or Lumason) and according to package insert instructions, including use of VialMix®, the device used to activate Definity. Administration will occur IV in coordination with sonography staff trained specifically in contrast ultrasound imaging.

5.5 Subject Compliance Monitoring

Since the study only involves a single visit for most subjects, there will be no issues with compliance and no role for compliance monitoring.

5.6 Prior and Concomitant Therapy

Comparison imaging studies (ultrasound and MRI) completed prior, concomitantly or post-CEUS will be collected.

5.7 Packaging

- Definity comes in single use 2-mL clear glass vials.
- Lumason comes in a clear vial containing 25 mg of powder along with one pre-filled syringe with 5 mL sodium chloride 0.9% diluent.

5.8 Blinding of Study Drug (if applicable)

Does not apply.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies at the Investigational Drug Service (IDS), an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.9.2 Storage

- Definity is stored between 2-8°C.
- Lumason is stored at 25°C; excursions permitted to 15-30°C.

5.9.3 Dispensing of Study Drug

Study drug (contrast agent) is not dispensed to the subject. It is used one-time, at the study visit and administered by study members.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Visit 1

After consent is signed, urinalysis and pregnancy test (if necessary) will be obtained. If the subject is cleared to proceed, IV access will be placed. Baseline B-mode US will be performed to confirm the lesions being imaged (unless it is a subject with no lesions, then a basic B-mode US will be performed). Contrast agent will be prepared by trained nursing staff. Once all study members and subject are prepared, contrast agent will be administered, and contrast enhancement of each lesion captured on a video clip. Once all lesions of interest are imaged, IV will be discontinued, and the subject discharged. Subject will be monitored throughout the study for any adverse events. See Flowchart (Appendix 1) for details.

Due to the uncommon occurrence of serious cardiopulmonary reactions, as listed in the package insert, resuscitation equipment and trained personnel will always be readily available during the study visit.

Subjects may be asked to return for an additional imaging visit the following 1-2 years for a repeat study to mirror clinical protocols for imaging in VHL.

7 Statistical Plan

7.1 Sample Size Determination

The goal of this feasibility study is to enroll a total of 15 subjects with familial syndrome at risk of kidney cancer, with at least 10 having 1 kidney lesion. This will yield up to 30 kidneys which will be assessed using CEUS, B-mode US and contrast MRI. Each of the 3 images on each kidney read by 3 readers with a Bosniak score assigned to each lesion. The primary objective is to assess agreement between the new imaging approach via CEUS with the most widely used existing technique, contrast-enhanced MRI, based on dichotomized Bosniak scores (I/II/IIF versus III/IV). A high kappa would warrant further investigation of CEUS as an alternative to MRI and other approaches. As described below in section 7.2, overall point estimates and confidence intervals will be computed for the kappa of interest based on averaging across the 3 readers. The proposed sample size will yield 95% confidence intervals with widths no greater than 0.30 over a wide range of true kappas, which should provide sufficient precision in kappa estimates to make a determination regarding feasibility of CEUS for future research.

7.2 Statistical Methods

Kappas will be calculated separately for each reader for CEUS versus B-mode US, CEUS versus contrast MRI, and B-mode US versus contrast MRI, using the dichotomized Bosniak score. Confidence intervals for kappas will be computed by bootstrapping subjects, accounting for potential correlations of kidneys within subjects. Differences in agreement between readers will be based on comparing reader specific kappas, with statistical significance of these differences tested via bootstrapping. An overall kappa will be computed for each paired imaging comparison by averaging kappas across readers, with a 95% interval calculated via bootstrapping. Differences in overall kappas for the 3 paired imaging comparisons will be assessed using a bootstrap test. The above analyses will also be done separately for kidneys with lesions and without lesions, to evaluate whether agreement varies in these two important subgroups. Agreement between readers will be determined separately for each imaging modality by computing kappas for all pairs of readers, along with bootstrap confidence intervals. An overall assessment of reader agreement for each of the 3 modalities will be calculated by averaging the pairwise kappas. Finally, weighted kappa analyses will be done using the raw 5-point Bosniak scores, similar to those for the dichotomized Bosniak scores.

7.3 Subject Population(s) for Analysis

All subjects will be used for analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- <u>Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***</u>

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

Not applicable as there is no study sponsor.

8.3.2 Investigator reporting:

For reportable deaths, the initial submission to the UNC IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 Investigator reporting:

Affiliate sites will inform study PI and primary site of any adverse events as described in sections 8.1, 8.2 and 8.3.

8.3.4 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
    -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

IND has not yet been submitted so the contact information is not yet available.

8.3.5 Sponsor reporting: Notifying participating investigators

This is a multi-center, investigator-initiated study.

8.4 Unblinding Procedures

Not applicable.

8.5 Stopping Rules

As this study is low risk to study subjects and involves a single visit and single vial of contrast agent for most subjects, stopping rules are not applicable. In the event of adverse event during contrast agent administration, injection will be immediately discontinued, and the subject treated appropriately (also described in section 4.4.1). Subject will be monitored for a minimum of 30 minutes following any possible adverse event related to contrast agent.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms (as applicable)

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by report of adverse events to a designated Medical Monitor every 6 months. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Appendix 2 for a copy of the Subject Informed Consent Form, Appendix 3 for a copy of the Assent Form for subjects aged 16 and 17 and Appendix 4 for a copy of the Parental Consent Form for subjects aged 16 and 17. These consent forms will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

Lantheus.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of North Carolina investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

There will be a subject payment of $50 and parking voucher for each study imaging visit.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol will be published or passed on to any third party without the consent of the principal investigator. The principal investigator holds the primary responsibility for publication of the results of the study.

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15 Appendices

Appendix 1

Study Procedures Table

	Screening	Pre-CEUS	CEUS study	Early Termination[1]	Post-CEUS study
Inclusion/ Exclusion Criteria	X				
Comprehensive Medical History	X	X[2]			
Informed Consent	X[3]	X[3]			
Education of potential side effects		X			
Pregnancy test (urine), if applicable		X[4]			
Urinalysis		X[5]	X		
Contrast-Enhanced US (CEUS)			X		
AE Assessment			X[6]	X	
Obtain any other imaging studies obtained during 4 months prior to or post study enrollment				X	X

[1]For 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.
[2]Comprehensive medical history obtained at time of enrollment; thereafter history focused on symptoms and assessments.
[3]Consent 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.
[4]Women of childbearing potential must have negative urine or serum pregnancy test on the day of CEUS study, prior to the study.
[5]Urinalysis will be obtained prior to and post CEUS study.
[6]Adverse event assessment will happen during the CEUS study when they will be monitored by 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 study PI's Office.