

**LCCC 1405: Comparison of the sensitivity and specificity of acoustic angiography (Micro-tumor detection by quantifying tumor-induced vascular abnormalities) to the sensitivity and specificity of conventional ultrasound**

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LINEBERGER COMPREHENSIVE CANCER CENTER  
CLINICAL ONCOLOGY RESEARCH PROGRAM  
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

**LCCC 1405: Comparison of the sensitivity and specificity of acoustic angiography (Micro-tumor detection by quantifying tumor-induced vascular abnormalities) to the sensitivity and specificity of conventional ultrasound**

**Principal Investigator**

Yueh Lee, MD, PhD  
University of North Carolina  
Department of Radiology  
919-537-3730 phone  
yueh\_lee@med.unc.edu

**Co-Investigators**

Cherie Kuzmiak, DO  
University of North Carolina  
Department of Radiology  
919-966-6392 phone  
cherie\_kuzmiak@med.unc.edu

Sheila Lee, MD  
University of North Carolina  
Department of Radiology  
sheila\_lee@med.unc.edu

**Biostatistician**

Donglin Zeng, PhD  
Department of Biostatistics  
University of North Carolina  
919-966-7273 – phone  
dzeng@bios.unc.edu

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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:** \_\_\_\_\_

**Version Date:** \_\_\_\_\_

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## 1.0 BACKGROUND AND RATIONALE

### 1.1 Study Synopsis

Increasing the sensitivity and specificity of diagnostic imaging in patients at high risk for breast cancer could provide substantial clinical benefit by improving diagnosis, preventing over-treatment, and reducing healthcare costs. Acoustic angiography is a new type of contrast enhanced ultrasound imaging which is specifically sensitive to microvascular structure and density. It evaluates tumor micro-vasculature and may provide a powerful prognostic tool for the diagnosis of breast cancer, and eventually for treatment evaluation.

Sixty patients who are to have a clinical surgical breast biopsy based on results from pre-study standard of care (SOC) imaging will be recruited from the UNC Breast Clinic for participation in the study. The primary objective of this single arm study is to compare the sensitivity and specificity of acoustic angiography with traditional b-mode ultrasound in the distinction of malignant versus benign breast lesions. Secondary objectives include a comparison of area under the curve (AUC) for acoustic angiography versus b-mode ultrasound, comparison of radiologist preference for the two imaging techniques for each of 3 lesion characteristics, and quantification of vessel tortuosity based on acoustic angiography imaging results. These metrics will be used to develop a predictive model of malignancy which will subsequently be compared to results from radiology review.

### 1.2 Traditional Breast Cancer Screening and Diagnosis

Breast cancer is the most common cancer type among women. Approximately 10% of women in the United States develop breast cancer during their lifetime and 30% to 40% of these patients will die from it.[33, 34] Mammography is an effective tool for the early detection of breast cancer in the majority of women.[35] However, for women with dense breast tissue (considered an independent risk factor for breast cancer) and younger women, mammography performs poorly due to lower sensitivity and specificity in these groups.[37] For young women with heritable mutations who wish to begin screening at a younger age, these limitations are especially problematic.[38] Additionally, mammography is less sensitive in women who have undergone breast augmentation. Given these limitations, most of these women may undergo additional imaging with breast ultrasound or Magnetic Resonance Imaging (MRI).

MRI is time consuming, extraordinarily costly, and has limited availability, especially among rural and underserved populations. Another screening option, breast ultrasound (without contrast), is widely used when additional imaging beyond mammogram is required due to its real-time imaging

capability (>30 images per second), portability, safety (does not involve radiation), and relatively low cost compared to breast MRI. Unfortunately, while breast ultrasound is highly sensitive (96%), it is less specific (70%), resulting in a high false positive rate. This results in unnecessary biopsies with associated complications, additional follow-up and negative psychosocial impacts on patients, e.g., significant anxiety. A significant clinical need exists to improve breast ultrasound sensitivity and specificity.

### 1.3 **Angiogenesis**

Angiogenesis is the development of new microvasculature, and is well recognized to be involved in the growth of solid tumors as well as tumor invasion and metastasis. Recent studies have reported an association between microvessel density and poorer recurrence-free, cancer-specific and overall survival.[39-46]. Furthermore, both microvessel density and microvessel morphology have been reported to be associated with the clinical response to chemotherapy.[16, 47-49] However, no current clinical imaging modality can directly evaluate the microvasculature associated with suspected breast tumors.[50] Histological techniques based on core needle biopsy or surgical biopsy may evaluate only a small portion of a lesion, and require additional invasive procedures. Thus, direct non-invasive evaluation of the tumor micro-vasculature may provide a powerful prognostic tool for the diagnosis of breast cancer, and also provide a potential tool for treatment evaluation.

#### 1.3.1 **Screening Based on Angiogenesis-Acoustic Angiography**

Acoustic angiography allows for the viewing of vessel shape and delineation of 'tortuosity,' which can indicate the presence and progression of cancer. This ability to image the microvasculature depends on the use of a multi (high) frequency ultrasound scanner in addition to a traditional single-frequency pulse-echo ultrasound scanner (b-mode ultrasound), the latter to ensure accurate anatomical location of the lesion. Because of this dependence on pulse-echo, the technique is referred to as "acoustic" angiography [62].

Acoustic angiography also depends on use of an ultrasound contrast agent. Contrast enhanced ultrasound imaging has been used for nearly two decades for clinical cardiology in the United States (and is currently routinely used in UNC Hospital Cardiology Clinics), and is much more widely used in Europe and Asia for visualization of blood perfusion in organs, tissues, and tumors.[51] Early concerns about the safety of ultrasound contrast (specifically with the contrast agent (perflutren lipid; Definity®) due to events in a clinical trial have been resolved; the overwhelming amount of more recent evidence from large clinical studies has shown that contrast ultrasound is very safe.[52-54] In fact ,it is much safer than other commonly used techniques, such as coronary angiography, exercise ECG, or myocardial scintigraphy.[55] Furthermore,

it does not have the risks of nephrogenic systemic fibrosis associated with gadolinium based MRI contrast agents when used in renally compromised patients. On October 27, 2011, the FDA eliminated the requirement for patient monitoring 30 minutes after ultrasound contrast examination, based on these and other recent safety data. The ultrasound contrast agent to be used in LCCC1405 is perflutren lipid.

#### **1.4 Perflutren Lipid Background and 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 6.1.5.

##### **1.4.1 Background and Current Indications**

Definity® (perflutren lipid) is an FDA-approved lipid-shell microbubble ultrasound (US) contrast agent that may be administered by an intravenous (IV) bolus or infusion. Currently, this contrast agent is approved for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve delineation of the left ventricular endocardial border. It is not approved as a contrast agent for acoustic angiography in the breast.

When used according to its approved indication, the maximum dose of perflutren is administered as either two bolus doses or one single intravenous infusion.

For our study, perflutren lipid will be administered intravenously by a nurse or trained medical personnel (see section 4.3).

##### **1.4.2 Associated Toxicities**

In pre-market clinical trials 1716 subjects were evaluated with activated perflutren lipid. Of the 1716 subjects, 144 subjects (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 eight 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*

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,[22] compared to gadolinium-based MRI contrast which has an incidence of NSF of 2 – 5 in 100 patients with chronic kidney disease.[23]

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 nurse or research physician.

#### *Hypersensitivity Reactions*

The real risk of perflutren in our study is to the small number of potential patients with 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 6.1.5 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)*

High ultrasound MI values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. In addition, end-systolic triggering with high mechanical indices has been reported to cause ventricular arrhythmias.

*Use in Patients with Known Breast Lesions*

US contrast agents including perflutren should carry no additional risks in patients with a known breast lesions, as they are cleared by the lungs. The phospholipid component of perflutren lipid microspheres are 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) .

**1.5 Rationale**

One main limitation to the widespread use of contrast enhanced ultrasound clinically has been the lack of availability of state-of-the art contrast imaging approaches available to clinicians. However, contrast ultrasound will likely become far more widespread as new imaging techniques, such as acoustic angiography, demonstrate their usefulness and become available on commercial ultrasound systems. We propose to evaluate a novel ultrasound method that could potentially improve the sensitivity and specificity of traditional breast ultrasound. Increasing the accuracy of diagnostic imaging in high risk patients could provide substantial clinical benefit by improving diagnosis, preventing over-treatment, and reducing healthcare costs.

LCCC1405 is designed to compare the sensitivity and specificity of contrast enhanced ultrasound (acoustic angiography) to the sensitivity and specificity of conventional ultrasound in women scheduled to undergo a biopsy based on pre-study imaging results. The gold standard for sensitivity and specificity, then, will be based on pathological results. The Breast Imaging Reporting and Data System (BIRADS) is used by radiologists who read mammograms, ultrasounds and MRIs to indicate their level of suspicion of the possibility of breast cancer. Scores range from 0 to 6, with scores of 4-5 indicating suspicious results, while 6 indicates an existing diagnosis of breast cancer. This study is limited to women with a score of 4-5 based on pre-study imaging.

## 2.0 STUDY OBJECTIVES AND ENDPOINTS

### 2.1 Primary Objective

To compare (using a reader study) the sensitivity and specificity of acoustic angiography to the sensitivity and specificity of conventional b-mode ultrasound in evaluation of breast lesions

### 2.2 Secondary Objectives

2.2.1 To compare the area under the curve (AUC) of acoustic angiography to the AUC of the b-mode ultrasound

2.2.2 To compare radiologist preference of acoustic angiography to conventional b-mode ultrasound for each lesion characteristic (shape, margins and vascularity)

2.2.3 To quantify vessel tortuosity metrics for the acoustic angiograph images, and to use these metrics to develop a model for predicting malignancy (a model-based malignancy score)

2.2.4 To compare the model-based malignancy score to the acoustic angiography reader study

### 2.3 Primary Endpoint

Sensitivity and specificity for our study is defined as the ability of readers (radiologists) to use the acoustic angiography or b-mode ultrasound to distinguish between malignant and non-malignant breast lesions known to exist based on pathological results (the gold standard).

## 3.0 ELIGIBILITY

### 3.1 Inclusion Criteria

Subject must meet all of the inclusion criteria to participate in this study:

3.1.1 Women  $\geq 18$  years old

3.1.2 Scheduled for breast core needle or surgical biopsy of at least one breast lesion based on suspicious breast lesion (BIRADS score of 4 or 5) from pre-study SOC imaging studies

3.1.3 Center of suspicious lesion is not deeper than 1.5 cm.

3.1.4 Able to provide informed consent

3.1.5 Negative urine pregnancy test in women of child-bearing potential

### 3.2 Exclusion Criteria

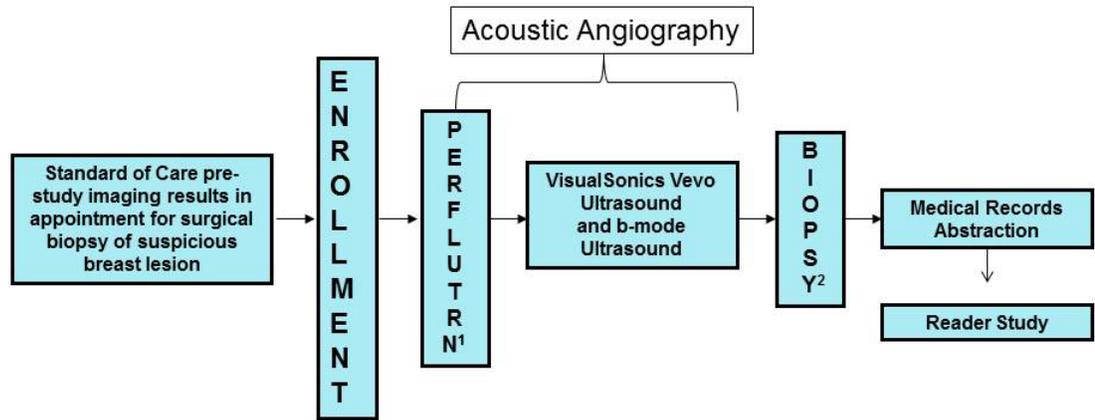
All subjects meeting any of the exclusion criteria at baseline will be excluded from study participation

- 3.2.1 Male (it is uncommon for men to present for imaging and the overwhelming majority of findings are non-cancerous and do not lead to biopsy; male breast cancer represents <1% of newly diagnosed breast cancer)
- 3.2.2 Institutionalized subject (prisoner or nursing home patient)
- 3.2.3 Critically ill or medically unstable and whose critical course during the observation period would be unpredictable (e.g., chronic obstructive pulmonary disease (COPD))
- 3.2.4 Known hypersensitivity to any component of perflutren lipid (Definity®)
- 3.2.5 Right to left shunt, severe pulmonary hypertension (pulmonary artery pressure >90mmHg), or adult respiratory distress syndrome
- 3.2.6 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.
  - Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; ventricular premature complexes occurring close to the preceding T-wave, multifocal complexes).
  - Myocardial infarction within 1 year prior to the date of proposed Definity® administration.
  - Uncontrolled systemic hypertension (systolic blood pressure (BP) >150 mm Hg and/or diastolic BP >90 mm Hg despite optimal medical management)
- 3.2.7 Any woman who is pregnant or has reason to believe she is pregnant or any woman who is lactating (the possibility of pregnancy has to be excluded by negative urine  $\beta$ -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)

## 4.0 STUDY PLAN

#### 4.1 Schema

This is a one arm single center study of 60 patients with suspicious breast lesions (BIRADS 4 or 5) from the UNC Breast Clinic that consent to undergo an acoustic angiography in conjunction with b-mode ultrasound prior to their scheduled biopsy.



<sup>1</sup> Pre-ultrasound contrast agent  
<sup>2</sup> Based on results of pre-study imaging

#### 4.2 Enrollment/Recruitment

A total of 60 women will be enrolled to this study. The 60 study subjects will be consecutively recruited from women who are scheduled to undergo core needle or surgical biopsy (BIRADS 4 or 5) to have pathological confirmation of malignancy status. Eligible patients will be identified by research staff review in coordination with the UNC Breast Clinic. Once a patient has been referred, the patient will be approached by a coordinator from Radiology to assess interest in participation.

All eligible women who agree to participate in the study will be asked to come to their scheduled biopsy appointment thirty minutes early to complete the informed consent process.

Review of the consent will take place in the privacy of an exam room, or when possible, a sample consent form will be sent to the patient via email prior to the patient's visit to allow for ample review. Once the patient has consented, women of child bearing potential (WCBP) will be given a urine pregnancy test in order to ensure that they are not pregnant. If a urine pregnancy test shows a result positive for pregnancy, the patient will be excluded from the study per the exclusion criteria because the investigators cannot, in good conscience, expose a fetus to the contrast agent used. Women who consent for the study and are eligible will be escorted by the research coordinator to a dressing room, where the subject will change into a gown.

### **4.3 Acoustic Angiography**

#### **4.3.1 Perflutren Administration**

At the time of imaging, the contrast agent perflutren lipid (see section 4.3) will be administered. See

[http://www.definityimaging.com/pdf/VIALMIX\\_Users\\_Guide.pdf](http://www.definityimaging.com/pdf/VIALMIX_Users_Guide.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. Cardiopulmonary resuscitation personnel and equipment will be readily available prior to perflutren administration, and all patients will be monitored for acute reactions.

Perflutren will be administered in a single dose using a bolus administration. (see <http://www.definityimaging.com/how-administration.html>). Although the prescribing information allows for two 10 uL/kg doses separated by 30 minutes, we will be administering double the package recommended dose (administering 20 uL/kg) administered within 30-60 seconds, followed by a 10mL saline flush. No second dose will be administered. All patients will be monitored for 30-minute post-perflutren administration by the research nurse or research physician. Monitoring will include taking vitals (O<sub>2</sub> sat, HR, RR, BP). The oxygen saturation, heart rate, and respiration rate will be monitored continuously for 30 min. The blood pressure will be monitored every 15 minutes. This study will be conducted in Mammography of the UNC Cancer Hospital, so trained medical personnel will be available as needed.

The initial concentration will be diluted, although the final dilution amount is not finalized (most likely to range from 1:1 (full dose) to - 1:5 dilution in saline). This dilution technique is still to be determined based on performance because this is a new type of imaging. We will infuse via hand bolus injection. The infusion time will be approximately 30 - 60 seconds for the dose.

#### **4.3.2 Acoustic Angiography Imaging Procedures**

Acoustic angiography imaging involves a research high frequency ultrasound scanner (VisualSonics Vevo 770; see section 5.0) in contrast imaging mode. In addition, we may use a standard FDA-approved clinical ultrasound system to obtain a second conventional B-mode ultrasound image as a gold standard. Imaging will be performed within the package insert guidelines for ultrasound system mechanical index (a measurement of output power) when imaging perflutren contrast agent (less than 0.8).

Acoustic angiography imaging will be performed by a trained medical personnel using mild compression to eliminate motion. A linear translation stage will be attached to the transducer to mechanically translate the

transducer the imaging distance (up to approximately 3 cm). Total imaging time per pass is estimated to be 60 seconds, similar to a standard ultrasonography exam. All image data will be de-identified and transferred for off-line analysis based on a study ID. The research images will NOT be interpreted or analyzed for clinical decisions related to the patient. See section 5.0 for additional information on the acoustic angiography device.

#### 4.4 Standard of Care Biopsy

The patient will then undergo her scheduled breast biopsy procedure based on the pre-study diagnostic imaging. The research acoustic angiography imaging will NOT be interpreted prior to the breast biopsy and therefore will not influence any clinical decision concerning the biopsy.

#### 4.5 Medical Record Abstraction

A secondary objective of this study is to quantify the vessel tortuosity and develop a “model-based malignancy score” to predict malignancy of a lesion based on quantitative tortuosity metrics from the acoustic angiography imaging. In order to meet this objective, we will review each patient’s clinical records, including their pathology report from biopsy. The malignancy will be determined as indicated by the pathology report.

#### 4.6 Reader Study

A total of five readers (radiologists trained in breast imaging) will be recruited to participate in evaluation of all imaging performed under LCCC1405.

In the reader preference study, each reader will be asked to compare the acoustic angiography case to the conventional b-mode ultrasound case to evaluate the imaging characteristics based on the BIRADS ultrasound lexicon

(<http://www.acr.org/~media/ACR/Documents/PDF/QualitySafety/Resources/BIRADS/USLexiconClass.pdf>). Specifically the relative ability to evaluate shape, margins and vascularity will be evaluated using a seven-point scale (-3 to +3) for the paired modality comparisons for each of the 60 cases.

The data collection form for the reader study portion is given in Appendix A, section 13.1.

The readers will be asked to assign a subjective malignancy score (-2 (highly not malignant) to +2 (highly malignant)) and their confidence for each lesion for each modality (0 to 100%). These will also be documented on the form in Appendix A. These scores will be used combined for a binary analysis. Malignancy scores of +1 and +2 will be considered malignant. Scores of -2, -1, and 0 will be considered not malignant. The confidence of malignancy will be used independently in the analysis.

### 5.0 Research Ultrasound Scanner: FUJIFILM VisualSonics Vevo 770

The device used for this study will be a research ultrasound scanner (FUJIFILM VisualSonics Vevo 770) and unique probes developed in conjunction with the research lab run by the founder of VisualSonics, Dr. Stuart Foster. The ultrasound probes are modifications of Vevo 770 probes (RMV716 probes), modified to house 2 single-element transducers instead of 1.

The Vevo 770 is a research ultrasound system designed for pre-clinical, high resolution imaging of small animals. It is marketed commercially by VisualSonics, Inc., a division of SonoSite, Inc., under the parent company FUJIFILM. This ultrasound system and similar models have been designated to pose no significant risk in other clinical trials by the respective IRBs or other similar regulatory agencies responsible for compliance oversight of these studies. (See section 13.1, Appendix B).

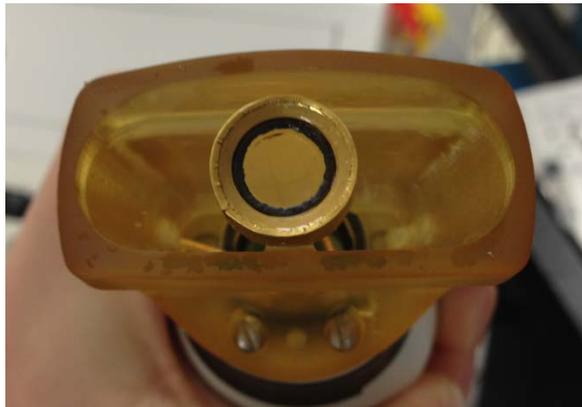
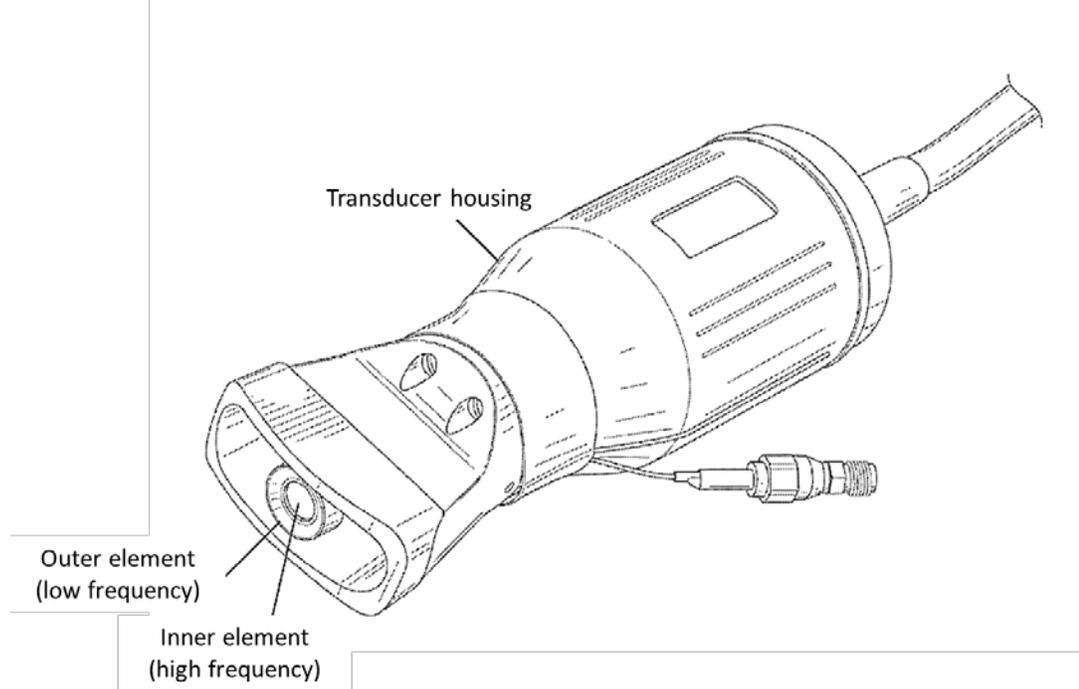


Figure 1. Device Image

### 3 Dual-Frequency Transducers:

The probes we plan to use in this study are modified VisualSonics RMV716 probes. They have been modified to house a high frequency inner element and a low frequency annular ring element, instead of a single transducer element. We plan to run the transducers in dual-frequency contrast mode during the study. In dual-frequency contrast mode, the low frequency (outer) element transmits ultrasound and the

high frequency (outer) element ring receives the signal. In this mode, the low frequency element is run by an external waveform generator and amplifier, and the high frequency element is run by the Vevo 770 system.



**Figure 2.** Device Transducers

## 5.1 Expected Risks

### 5.1.1 VisualSonics Vevo 770

This research protocol presents minimal risk to participants, investigators and study personnel. The ultrasound imaging equipment, though specifically designed for preclinical use and not FDA approved, is calibrated and will be used according to FDA limits on diagnostic imaging ultrasound exposure to patients (Mechanical Index of less than 1.9 and spatial-peak temporal average intensity of less than 720 mW/cm<sup>2</sup>). For

the imaging studies proposed here, the maximum mechanical index and intensity will not exceed 0.8 and 300 mW/cm<sup>2</sup>, respectively, less than half of the FDA limits, and within the range of standard output parameters regularly used in diagnostic ultrasound imaging. Furthermore, this is within the mechanical index range listed on the prescribing information for use of perflutren contrast agent. The 1.5 cycle pulse length and 2-4 MHz center frequency of the ultrasound that is produced by our transducers are within the range of common parameters regularly used by other FDA approved diagnostic ultrasound imaging systems. The unique aspect of this imaging system is that we receive data at a higher frequency (15-45 MHz) than typical diagnostic ultrasound imaging systems used for transcutaneous imaging. The transmitted ultrasound parameters are within standard diagnostic imaging ranges for frequency and acoustic output.

### 5.1.2 Perflutren lipid (Definity)

See sections 1.4 and 6.1.5.

## 5.2 Duration of Study

It is anticipated that the total study duration encompassing recruitment, enrollment, and data analysis will take approximately 2 years. Active patient participation will last approximately 1 visit (consent and 15 minutes imaging).

## 6.0 Drug Information

### 6.1 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 for use 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 x 10<sup>10</sup> perflutren lipid microspheres with approximately 150 µL/mL octafluoropropane.

### 6.1.1 Supplier/How Supplied

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.

### 6.1.2 Handling and Dispensing of Perflutren

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.

### 6.1.3 Storage Requirements/Stability

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.

### 6.1.4 Preparation

See [http://www.definityimaging.com/pdf/VIALMIX\\_Users\\_Guide.pdf](http://www.definityimaging.com/pdf/VIALMIX_Users_Guide.pdf), and the package insert for instructions on the use of Vialmix®.

### 6.1.5 Clinical Safety Summary

See prescribing information on perflutren when used according to its FDA indication (<http://www.accessdata.fda.gov/scripts/cder/drugsatfda/>), and see section 1.3 for a summary of toxicities reported in clinical trials. In addition, the following warnings and precautions are noted in the October 2011 labeling:

#### *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.

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.

#### *Anaphylactoid Reactions:*

In post-marketing 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 have occurred in patients with no prior exposure to perflutren-containing microsphere products.

*Systemic Embolization of Perflutren in Patients with Cardiac Shunts:*

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 15 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:*

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:*

ECG parameters for doses up to 10 microL/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.

**6.1.6 Return and Retention of Study Drug**

Incomplete vials of perflutren lipid remaining at the completion of the study, or expired perflutren lipid will be destroyed by UNC IDS.

**7.0 ADVERSE EXPERIENCES-DRUGS**

## 7.1 Definitions

### 7.1.1 Adverse Event (AE)

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.

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)

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.

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)
- 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 once 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.
- 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

An AE or SAR is considered unexpected if the sensitivity and 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

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:

- 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

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30 day follow-up period after treatment is discontinued.

Collected information should be recorded in the electronic Case Report Forms (e-CRF) for that patient. Please include a description of the event,

its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

### **7.3 SAEs or Serious SARs**

#### **7.3.1 Timing**

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout, or no treatment run-in).

For any other 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**

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

#### **7.3.3 Reporting**

##### **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.
- 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 8.2 ) within 7 days of the Investigator becoming aware of the problem.

##### Pregnancy

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.

## 8.0 UNANTICIPATED CONCERNS

### 8.1 Unanticipated Adverse Device Effect (UADE)

The investigational device exemption (IDE) regulations define an unanticipated adverse device effect (UADE) as “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects” (21 CFR 812.3(s)).

### 8.2 Unanticipated Problems (UP)

As defined by UNC’s IRB, unanticipated problems involving risks to study subjects refers to any incident, experience, or outcome that:

- Is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Is related or possibly related to a subject’s participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

### 8.3 Reporting

#### 8.3.1 UADEs

UADEs must be reported by the clinical investigator to the sponsor and the reviewing IRB, as described below:

For this device study, investigators are required to submit a report of a UADE to the FDA, the manufacturer of the device and the UNC IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event (§ 812.150(a)(1)), using the MedWatch Form 3500A. Sponsors (LCCC) must immediately conduct an evaluation of a UADE and must report the results of the evaluation to FDA, the UNC IRB, and participating investigators within 10 working days after the sponsor first receives notice of the effect (§§ 812.46(b), 812.150(b)(1)).

For this device study, we will submit a report of a UADE to the manufacturer and the IRB as soon as possible, but no later than 10 working days after the investigators first learn of the event.

### 8.3.2 UP

Any events that meet the criteria for “Unanticipated Problems” as defined by UNC’s IRB must be reported by the Study Coordinator using the IRB’s web-based reporting system.

Any unanticipated problem that occurs during the conduct of this study and that meets **at least** the first two criteria listed in section 8.2 must be reported to the UNC IRB using the IRB’s web-based reporting system.

## 9.0 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.

## 10.0 STATISTICAL CONSIDERATIONS

This is a nonrandomized, single-center study. The primary purpose and endpoint of this study is to compare, in a radiologist reader study, the sensitivity and specificity of the acoustic angiography system to the b-mode ultrasound with pathology as the reference standard.

### 10.1 Sample Size and Accrual

Per the revision in increased Perflutren dose, we have updated our sample size statistics as follows.

For power calculation, the null hypothesis is that the specificity of acoustic angiography system is the same as the standard of care (b-mode ultrasound), which is assumed to be 70%. (We will determine the specificity of b-mode ultrasound during the trial to confirm that the 70% represents the specificity in the hands of UNC radiologists) Under the alternative, we expect that the specificity of the new device is at least 90%. The specificities are measured relative to pathological diagnosis as the reference standard. With 60 lesions and 5 readers, where we anticipate that roughly half will be malignant, assuming the correlation from the same patient to be 0.5 and the readers to read different patients independently, the power to see specificity at least larger than 90% is 98% at the significance level 0.05 using one-side test. With 50 lesions (half are benign), the power to see specificity of 90% is >96%; with 25 lesions (about 13 are benign), this power becomes 72%. The power to reject the null hypothesis is 98%. Although we will study both sensitivity and specificity, our power calculation is based on specificity, which is the primary interest.

### 10.2 Data Analysis Plans

#### 10.2.1 Primary Analysis

This is a prospective study to assess the diagnostic performance of the newly developed device. The **primary outcome of interest is the sensitivity and specificity of the acoustic angiography device as compared to b-mode ultrasound**. The sensitivity and specificity can be estimated non-parametrically as the proportion of the lesions which are distinguished as malignant versus non-malignant compared to the reference. This estimate will be calculated for each reader then averaged over all the readers. The standard error of the average sensitivity and specificity estimate will be calculated using the bootstrap method, where each patient is treated as independent unit with 5 ratings. The confidence interval of the final estimate will be provided using the normality assumption.

Furthermore, we will estimate the sensitivity and specificity of the b-mode ultrasound in this study and compare it with the sensitivity and specificity estimate of the acoustic angiography device using the bootstrap approach and the confidence interval of their differences will be provided using the normality assumption.

Malignancy scores of +1 and +2 will be considered malignant. Scores of -2, -1, and 0 will be considered not malignant. The confidence of malignancy will be used independently

### 10.2.2 Secondary Analyses

#### Radiologist Preference

The secondary analysis will be to estimate the receiver operating characteristic (sensitivity and specificity) curve for the acoustic angiography system, with an additional aim of evaluating reader preference for specific breast lesion characteristics. Specifically, to compare the diagnostic performance, we will non-parametrically calculate the area under the ROC curve for each reader and each modality, where the ROC curve is derived using the different cut-off of the probability scores across 60 patients. We then fit a mixed effect model, where the outcomes are the estimated area under the ROC curves and the fixed effect is the dummy variable of the acoustic angiography system vs. conventional b-mode ultrasound. The readers will be treated as random effect. F-test from model fitting will be used to test whether the acoustic angiograph has a significant larger AUC than the conventional b-mode ultrasound (with significance level 0.05).

To assess the reader preference for modality for each characteristic including shape, margins and vascularity, we will fit a random effect model with only intercept and random terms for patients and readers while the outcomes are the confidence scores (-3 to +3) . By testing the intercept significantly larger than zero, we will conclude that the new modality provides more confidence for readers than the conventional one.

#### Image Segmentation and Tortuosity Quantification

In order to derive quantitative vessel tortuosity metrics for the acoustic angiography images, we will perform an image processing workflow. The de-identified DICOM images will be converted to a 3-D volume and linearly up-sampled using MATLAB to create isotropic 50 x 50 x 50 micron voxels. Then, the lesion will be manually segmented to identify a region of interest (ROI) for vessel analysis. Using a series of algorithms [59, 60], each vessel in the ROI will be segmented and a series of tortuosity metrics will be calculated. The vessels are segmented using semi-automated multi-scale ridge traversal and stored as a series of center-line point coordinates (x,y,z) forming a spline. The radius of the vessel is estimated at each point and saved as a fourth parameter. [14, 61] Each

tortuosity metric will be used individually to contrast benign and malignant lesions using a two-sided t-test, incorporating a Bonferroni correction to account for multiple hypothesis testing.

#### Predicting Malignancy

In order to predict malignancy based on the quantitative vessel tortuosity metrics, the set of images will be randomly divided into two halves to form a training data set and a test data set. The malignant lesions and the benign lesions will be randomly assigned to the training or test group separately to ensure that both groups contain both types of images. Multivariate binary logistic regression will be used to predict malignancy based on the full set of tortuosity metrics for the training data set of 30 images. We will examine the model for multicollinearity using pairwise correlations and perform linear discriminant analysis to choose a smaller subset of metrics if over-fitting due to multicollinearity appears to be a problem. The model will then be used to predict the probability of malignancy of the test set of 30 images (via development of a model-based malignancy score). We will construct a receiver operator curve (ROC) by determining the sensitivity and specificity of the model when varying the threshold of probability of malignancy. We can then compare the performance of the quantitative tortuosity model to the reader study by comparing the area under the curve for both ROCs.

## 11.0 STUDY MANAGEMENT

### 11.1 Institutional Review Board (IRB) Approval and Consent

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) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto 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.

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.

## **11.2 Required Documentation**

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

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- A copy of the IRB-approved consent form
- Executed clinical research contract (if applicable)

The above documentation will be provided to our Study Sponsor (LCCC).

## **11.3 Registration Procedures**

Patients will be registered into OnCore®, a web based clinical research platform by one of the Study Coordinators. The spread sheet contains each subject enrolled in the study identified by the patient first and last initial, study id, date of enrollment into study, race and ethnicity.

## **11.4 Data Management and Monitoring/Auditing**

The breast images of all eligible enrolled subjects that are obtained and contribute to the ultimate diagnosis leading to biopsy will be de-identified for inclusion in the reader study. Copies of the clinical report forms as well as the de-identified images described in the preceding will be submitted for each case to the Study Coordinators for maintaining the study record and entering the data into a spreadsheet in preparation for the reader study.

As an investigator initiated study, this trial may also be audited by the Lineberger Cancer Center audit committee every twelve months.

## **11.5 Adherence to the Protocol**

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.

### **11.5.1 Emergency Modifications**

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC IRB approval.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

### 11.5.2 Single Patient/Subject Exceptions

Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the UNC IRB.

### 11.5.3 Other Protocol Deviations/Violations

All other planned deviations from the protocol must have prior approval by the Principal Investigator and the UNC IRB. According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- 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:

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

If a deviation or violation occurs without prior approval from the Principal Investigator, please follow the guidelines below:

**Protocol Deviations:** UNC personnel will keep a log of any protocol deviations and report them to the study 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.

**Protocol Violations:** Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

### 11.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. 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 UNC's IRB for approval prior to implementation.

### **11.7 Record Retention**

Study documentation includes all Case Report Forms, 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.

### **11.8 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/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. 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 case report forms 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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### 13.0 APPENDICES

#### 13.1 Appendix A: Reader Study Data Collection Form

Specimen: \_\_\_\_\_

Reader: \_\_\_\_\_

Date: \_\_\_\_\_

#### Overall Assessment:

##### Acoustic Angiography

Overall specimen malignancy score (-2 to +2): \_\_\_\_\_

Malignancy confidence (0-100) \_\_\_\_\_%

##### ULTRASOUND

Overall specimen malignancy score (-2 to +2): \_\_\_\_\_

Malignancy confidence (0-100) \_\_\_\_\_%

#### 1. Acoustic Angiography versus ULTRASOUND Shape/Morphology

|    |  |
|----|--|
| +3 | I am significantly more confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to <b>ULTRASOUND</b> representation of the same lesion      |
| +2 | I am more confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to <b>ULTRASOUND</b> representation of the same lesion                    |
| +1 | I am slightly more confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.      |
| 0  | I have the same confidence in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as I do in the <b>ULTRASOUND</b> representation of the same lesion             |
| -1 | I am slightly less confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.      |
| -2 | I am less confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.               |
| -3 | I am significantly less confident in the <b>Acoustic Angiography</b> representation of the lesion shape/morphology I described as compared to the <b>ULTRASOUND</b> representation of the same lesion. |

**2. Acoustic Angiography versus ULTRASOUND: Vascularity**

|    |   |
|----|---|
| +3 | I am significantly more confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to the <b>ULTRASOUND</b> representation of the same lesion. |
| +2 | I am more confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to <b>ULTRASOUND</b> representation of the same lesion                    |
| +1 | I am slightly more confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.      |
| 0  | I have the same confidence in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as I do in the <b>ULTRASOUND</b> representation of the same lesion.            |
| -1 | I am slightly less confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.      |
| -2 | I am less confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.               |
| -3 | I am significantly less confident in the <b>Acoustic Angiography</b> representation of the lesion vascularity I described as compared to the <b>ULTRASOUND</b> representation of the same lesion. |

**3. Acoustic Angiography versus ULTRASOUND Margins /Distribution**

|    |  |
|----|--|
| +3 | I am significantly more confident in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as compared to <b>ULTRASOUND</b> representation of the same lesion      |
| +2 | I am more confident in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as compared to <b>ULTRASOUND</b> representation of the same lesion                    |
| +1 | I am slightly more confident in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.      |
| 0  | I have the same confidence in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as I do in the <b>ULTRASOUND</b> representation of the same lesion             |
| -1 | I am slightly less confident in the <b>Acoustic Angiography</b> representation of the lesion <b>ULTRASOUND</b> representation of the same lesion.  |
| -2 | I am less confident in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as compared to the <b>ULTRASOUND</b> representation of the same lesion.               |
| -3 | I am significantly less confident in the <b>Acoustic Angiography</b> representation of the lesion margins/distribution I described as compared to the <b>ULTRASOUND</b> representation of the same lesion. |

### 13.1 Appendix B: Previous Clinical Investigation Use of Device:

The Vevo 770 investigational device and its successor the Vevo 2100 have been utilized in a number of clinical research studies deemed non-significant risk by the respective IRB or other regulatory agency responsible for human subject safety.

#### UNITED STATES

- University of Louisville, Louisville: Novel Pediatric Applications of High Resolution Ultrasound Imaging. This study evaluates the Vevo 2100 in comparison with standard ultrasound in the imaging of head, heart, and kidneys in premature infants.
- Jewish Hospital and St. Mary's Healthcare, Louisville: High Resolution Ultrasound in the Study of Arteriopathy in Composite Tissue Allotransplantation of the Hand. This study utilizes the Vevo 2100 to monitor blood flow, vessels, arteries and artery intima thickness, for signs of potential allograft rejection.
- Hospital of the University of Pennsylvania, Philadelphia: High-Frequency Ultrasound for Evaluation of Intimal Thickness. This study utilized a Vevo 770 to measure the intima separately from the media in the brachial, radial, and anterior tibial arteries.
- University of Pittsburgh Medical Center, Pittsburgh: Non-invasive High Resolution Ultrasound Evaluation. This study utilizes a Vevo 2100 to upper extremity arterial vasculature.

#### CANADA

- St. Michael's Hospital, Toronto (ITA 123218): Maturation of the Arterio-venous Fistula for Hemodialysis. This study utilizes the Vevo 770 to track the AVF maturation process by monitoring of the vascular remodeling of the blood vessels, to better predict AVF readiness for cannulation.
- Hospital for Sick Kids, Toronto (ITA 137454): Very High-Resolution Vascular Imaging in Children with Normal and Abnormal Vascular Development and Maturation. This study utilizes the Vevo 770 to determine the vascular function of peripheral arteries in children with or without abnormal pre- and post-natal hemodynamics.
- Mt. Sinai Hospital, Toronto (ITA 163547): High-Resolution Micro Ultrasound in the Diagnosis and Treatment of Male Infertility. This study utilizes the Vevo 2100 to establish normal parameters for the human testes using high resolution ultrasound to demonstrate its diagnostic advantage over standard ultrasound.

## **NETHERLANDS**

- Erasmus Medical Center, Rotterdam: High-Resolution Ultrasonography of the Cutaneous Nerve Branches in the Hand and Wrist. This study utilized the Vevo 770 to image the median, radial, ulnar, and digital nerves to evaluate the Vevo in the diagnosis of various neuropathies, including nerve injury.
- Erasmus Medical Center, Rotterdam: Radial Access Research: Echo Based Radial Artery Evaluation for Diagnostic or Therapeutic Coronary Intervention. This study utilizes the Vevo 2100 to assess arterial wall healing following radial artery cannulations.

## **SWEDEN**

- University Hospital, Uppsala: Thicker Carotid Intima Layer and Thinner Media Layer in Subjects with Cardiovascular Diseases. This study utilizes the Vevo 2100 to evaluate vascular risk in connection with systemic lupus erythematosus (SLE) and hormone replacement therapy.
- Sahlgrenska University Hospital, Goteborg: High-Resolution Ultrasound Showing Increased Intima and Media Thickness of the Radial Artery in Patients with End-Stage Renal Disease. This study utilized the Vevo 770 to measure intima and media thickness separately in patients with end-stage renal disease in order to investigate the radial arterial wall layers in patients with chronic renal failure.
- Sahlgrenska University Hospital, Goteborg: Increased Intima Thickness of the Radial Artery in Patients with Coronary Heart Disease. This study utilized the Vevo 770 to assess intima thickness separately from intima-media thickness as a potential tool for non-invasive early detection of atherosclerosis.
- Sahlgrenska University Hospital, Goteborg: Increasing Peripheral Artery Intima Thickness from Childhood to Seniority. This study utilized the Vevo 770 in carotid, radial and anterior tibial arteries, measuring intima thickness and intima-media thickness to establish that intima thickness-to-lumen diameter ratio increases with age.