LCCC 1748: Academic-Industrial Partnership for Translation of Acoustic Angiography

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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:_____

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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, and comparison of radiologist preference for the two imaging techniques for each of 3 lesion characteristics.

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 LCCC1748 is perflutren lipid. An IND exemption will be requested from the FDA for the usage of perflutren prior to usage in this study.

1.4 Perflutren Lipid Background and Known Toxicities

See http://www.definityimaging.com/pdf/DEFINITY_US_PI_515987-0117.pdf 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.

LCCC1748 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 known breast lesions for predicting malignancy.

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)

2.3 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 ≥18 years old

3.1.2 Patient had a diagnostic breast ultrasound study performed at UNC

3.1.3 Scheduled for a core needle or surgical breast biopsy of at least one breast lesion that is 2 cm or less in size and 3 cm in depth from the skin surface

3.1.4 Lesion visualized on ultrasound

3.1.5 Able to provide informed consent

3.1.6 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 Sonographically visible breast lesion larger than 2cm or greater than 3cm in depth from the skin surface

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

3.2.6 Active cardiac disease including any of the following:

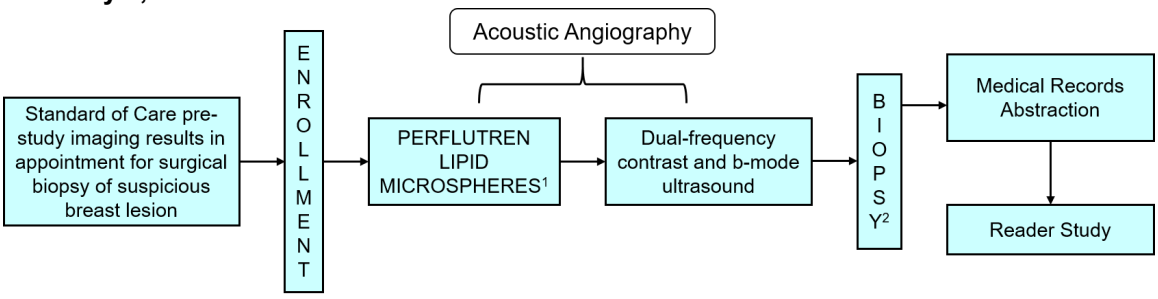
- Severe congestive heart failure (class IV in accordance with the classification of the New York Heart Association)
- Unstable angina.
- Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; ventricular premature complexes occurring close to the preceding T-wave, multifocal complexes).
- Myocardial infarction within 14 days 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 β-HCG results, obtained within 24 hours before the perflutren lipid administration, or on the basis of patient history, as defined by the UNC IRB SOP 4801.)

4.0 STUDY PLAN

4.1 Schema

This is a one arm single center study of 60 patients scheduled to be biopsied from the UNC Breast Clinic that consent to undergo an acoustic angiography in conjunction with b-mode ultrasound prior to their scheduled biopsy.



[1]Pre-ultrasound contrast agent
[2]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 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, 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 split doses using the dosing range and administration type IV bolus) within the perflutren prescribing information (see http://www.definityimaging.com/how-administration.html). When administered as a bolus, the package insert recommends 10 uL/kg patient weight administered within 30-60 seconds, followed by a 10mL saline flush with a second dose of 10 uL/kg patient weight 30 minutes following the first dose, if needed. All patients will be monitored for 15-minute post-perflutren administration by the research nurse or research physician. Monitoring will include taking vitals (O_2 sat, HR, RR, BP). The oxygen saturation, heart rate, and respiration rate will be monitored continuously for 15 min. The blood pressure will be monitored at 15 minutes. This study will be conducted in Mammography of the UNC Cancer Hospital, so trained medical personnel will be available as needed.

We will infuse via hand bolus injection for a "precision controlled bolus".

4.3.2 Acoustic Angiography Imaging Procedures

Acoustic angiography imaging involves a research ultrasound scanner (Verasonics Vantage 256; see section 5.0) as well as conventional b-mode ultrasound to guide the location of the imaging. The conventional ultrasound will be conducted just prior to the acoustic angiography for localization. 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. Total imaging time is estimated to be less than 15 minutes. 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

The primary objective of this study is to determine the sensitivity and specificity of 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 LCCC1748.

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 order that the modalities will be presented to the radiologists are randomized. The first modality presented for the specimen will be considered the primary and the second modality presented will be considered the secondary for the survey instrument. After a washout period of four weeks, the modalities will be presented in reverse.

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: Verasonics Vantage 256

The device used for this study will be a programmable research ultrasound scanner (Vantage 256, Verasonics, Inc., Kirkland, WA, USA) and a unique probe developed in conjunction with the research lab run by Dr. Stuart Foster (Sunnybrook Research Institute, Toronto, ON, Canada) and VisualSonics (FUJIFILM VisualSonics, Inc., Toronto, ON, Canada). The ultrasound probe developed is a modification of a preclinical high frequency transducer, modified to house a dual-frequency linear array.

The Vantage 256 is a programmable research ultrasound system. It is marketed commercially by Verasonics, Inc. For this study, two Vantage 256 platforms will be used. Each system will be programmed for

diagnostic ultrasound imaging at acoustic exposures similar to those approved for clinical use. 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 when acoustic output is maintained at or below FDA-approved levels (see section 13.1, Appendix B).

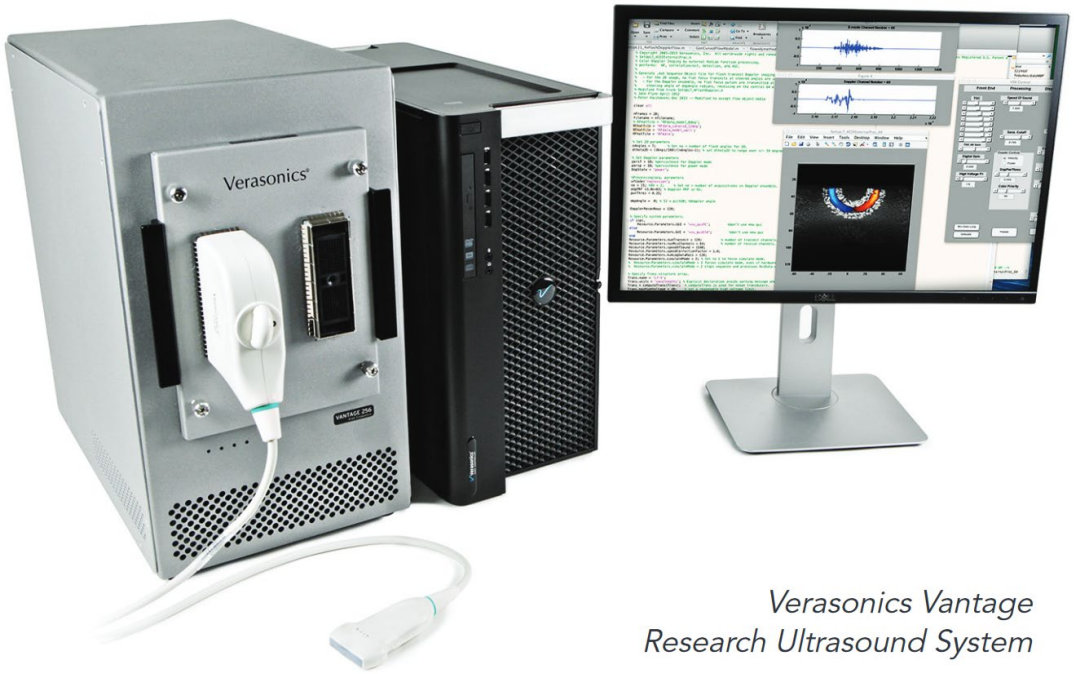


Figure 1. Device Image

Dual-Frequency Transducers:
The probes we plan to use in this study are modified VisualSonics probes. They are dual-frequency linear transducer arrays. We plan to run the transducers in 2 modes during the study: b-mode and dual-frequency contrast mode. In b-mode, only the high frequency elements are transmitting and receiving ultrasound. B-mode imaging will be performed using a designated b-mode program on one Vantage 256 scanner. In dual-frequency contrast mode, the low frequency elements transmit ultrasound and the high frequency elements receive the signal. To do so, one Vantage 256 controls the low frequency elements, and a second Vantage 256 controls the high frequency elements. Operation of the systems is synchronized and controlled by one designated dual-frequency program. It should be noted that only *one* Vantage system will be transmitting at any given time. The center frequency is 2 MHz for the low frequency elements and 20 MHz for the high frequency elements.

5.1 Expected Risks

5.1.1 Verasonics Vantage 256

This research protocol presents minimal risk to participants, investigators and study personnel. Our preliminary experimental and theoretical data show that the proposed stack design does effectively preserve excellent independent transmission of the two beams from the same aperture. Additional FEM modeling will be conducted to look at minimizing HF pulse distortion by varying the interlayer thicknesses and acoustic properties. The LF device will be designed to produce pulses with a mechanical index below the FDA limit of 1.9. Materials and processes will be adjusted to ensure that the probe temperature does not increase more than 6°C, as stated by the FDA.[52] Thermal effects will also be modeled using finite element methods (PZFlex). Analysis of the low frequency beam will be performed to optimize the number and lateral extent of low frequency elements. The current transducer design contains 32 LF elements, which we believe should be enough in the low frequency array. Based on simulations and experimental findings, however, future transducer iterations may extend this to 64 elements for improved imaging performance. Although the high frequency array by itself has been previously optimized with respect to its use in preclinical VisualSonics systems, the dual-frequency variant and effects of the various polymer and metal layers within flex circuits required for element connectivity will need to be included within the acoustic modeling of the transducer stack.

5.2 Perflutren lipid (Definity)

See sections 1.4 and 6.1.5.

5.3 Duration of Study

It is anticipated that the total clinical study duration encompassing recruitment, enrollment, and data analysis will take approximately 2 years and will begin in year 4 of the grant. 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 1.2×10^{10} 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, 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.definityimaging.com/pdf/DEFINITY_US_PI_515987-0117.pdf), 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 January 2017 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 DEFINITY administration and monitor all patients for acute reactions. The reported reactions include: fatal cardiac or respiratory arrest, shock, syncope,

symptomatic arrhythmias (atrial fi brillation, tachycardia, bradycardia, supraventricular tachycardia, ventricular fi brillation, ventricular tachycardia), hypertension, hypotension, dyspnea, hypoxia, chest pain, respiratory distress, stridor, wheezing, loss of consciousness, and convulsions. *Hypersensitivity Reactions:*

In postmarketing use, serious hypersensitivity reactions were observed during or shortly following perfl utren-containing microsphere administration including: Shock, bronchospasm, throat tightness, angioedema, edema (pharyngeal, palatal, mouth, peripheral, localized), swelling (face, eye, lip, tongue, upper airway), facial hypoesthesia, rash, urticaria, pruritus, fl ushing, and erythema have occurred in patients with no prior exposure to perfl utren-containing microsphere products. Always have cardiopulmonary resuscitation personnel and equipment readily available prior to DEFINITY administration and monitor all patients for hypersensitivity reactions.

Systemic Embolization of Perflutren in Patients with Cardiac Shunts:

When administering DEFINITY to patients with a cardiac shunt, the microspheres can bypass fi ltering by the lung and enter the arterial circulation. Assess patients with shunts for embolic phenomena following DEFINITY administration. DEFINITY is only for intravenous administration; do not administer DEFINITY by intra-arterial injection.

Ventricular Arrhythmia Related to High 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. DEFINITY is not recommended for use at mechanical indices greater than 0.8..

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

10.1.1 Primary analysis

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 88% (the variance of the estimated specificity is calculated to be less than 0.6*0.25/30) at the significance level 0.05 using one-side test. Although we will study both sensitivity and specificity, our power calculation is based on specificity.

10.1.2 Secondary Analysis

Sixty (60) women scheduled to undergo breast biopsy based on an abnormal ultrasound finding will be enrolled. We assume the AUC for the b-mode ultrasound images is 60% with range 60%~80%, and AUC difference of 10%. Assuming that the prevalence of noncancerous lesions in the sample population will be approximately 50%, the study will be powered at least at 80% with 56 patients and 5 readers.

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.

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

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

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:
Primary
Overall specimen malignancy score (-2 to +2): _____
Malignancy confidence (0-100) _____%

Secondary
Overall specimen malignancy score (-2 to +2): _____
Malignancy confidence (0-100)_____%

1. Primary versus Secondary Shape/Morphology

+3	I am significantly more confident in the **Primary** representation of the lesion shape/morphology I described as compared to **Secondary** representation of the same lesion
+2	I am more confident in the **Primary** representation of the lesion shape/morphology I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion shape/morphology I described as I do in the **Secondary** representation of the same lesion
-1	I am slightly less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.

2. Primary versus Secondary: Vascularity

+3	I am significantly more confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
+2	I am more confident in the **Primary** representation of the lesion vascularity I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion vascularity I described as I do in the **Secondary** representation of the same lesion.
-1	I am slightly less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.

3. Primary versus Secondary Margins /Distribution

+3	I am significantly more confident in the **Primary** representation of the lesion margins/distribution I described as compared to **Secondary** representation of the same lesion
+2	I am more confident in the **Primary** representation of the lesion margins/distribution I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion margins/distribution I described as I do in the **Secondary** representation of the same lesion
-1	I am slightly less confident in the **Primary** representation of the lesion **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.

13.1 Appendix B: Previous Clinical Investigation Use of Device:

The Verasonics Vantage investigational device and its predecessors 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

- Duke University, Durham, NC: Improved Visualization of Endocardial Borders with Short-Lag Spatial Coherence Imaging of Fundamental and Harmonic Ultrasound Data. DOI: 10.1109/ULTSYM.2012.0531. This study used a Verasonics scanner to implement a new imaging scheme to improve endocardial delineation.
- University of Washington, Seattle, WA: Ultrasonic propulsion of kidney stones: preliminary results of human feasibility study. DOI: 10.1109/ULTSYM.2014.0126. This study utilized a Verasonics system to perform ultrasonic propulsion of kidney stones as an alternative to surgery.
- Mayo Clinic College of Medicine, Rochester, MN: Pediatric Cardiac Shear Wave Elastography for Quantitative Assessment of Myocardial Stiffness: A Pilot Study in Healthy Controls. DOI: 10.1016/j.ultrasmedbio.2016.03.009. This study implemented shear wave elastography on a Verasonics Vantage system to evaluate myocardial stiffness in children.
- Mayo Clinic College of Medicine, Rochester, MN: Comb-Push Ultrasound Shear Elastography (CUSE) for Evaluation of Thyroid Nodules: Preliminary In Vivo Results. DOI: 10.1109/TMI.2014.2346498. This study used a Verasonics platform to measure tissue stiffness of benign and malignant thyroid nodules with elastography.
- Mayo Clinic College of Medicine, Rochester, MN: Probe Oscillation Shear Wave Elastography: Initial In Vivo Results in Liver. DOI: 10.1109/TMI.2017.278085. This study employed a Verasonics system to perform a novel elastography technique in the liver.
- Mayo Clinic College of Medicine, Rochester, MN: Effect of Calcifications on Breast Ultrasound Shear Wave Elastography: An Investigational Study. DOI: 10.1371/journal.pone.01378. This study used a Verasonics scanner to evaluate the results of shear wave elastography when breast calcifications are present.
- Columbia University, New York, NY: Evaluation of Coronary Artery Disease Using Myocardial Elastography with Diverging Wave Imaging: Validation Against Myocardial Perfusion Imaging and Coronary Angiography. DOI: 10.1016/j.ultrasmedbio.2017.01.001. This study compared a

novel elastography technique on a Verasonics system to non-ultrasound standard of care imaging methods used for the assessment of coronary artery disease.

CANADA

- University of Montreal Hospital, Montreal, QC: High-Frame-Rate Echocardiography Using Coherent Compounding with Doppler-Based Motion-Compensation. DOI: 10.1109/TMI.2016.2523346. This study utilized a Verasonics scanner to implement motion-corrected echocardiography at high frame rates.
- University of Montreal Hospital, Montreal, QC: High-Frame-Rate Speckle-Tracking Echocardiography. DOI: 10.1109/TUFFC.2018.2809553. This study used a Verasonics system to assess a novel image processing technique on echocardiography images.

EUROPE

- Imperial College, London, UK: High Frame-Rate Contrast Echocardiography: In-Human Demonstration. DOI: 10.1016/j.jcmg.2017.09.011. This study used a Verasonics scanner to demonstrate an improvement in contrast echocardiography by using high frame rate imaging.