THOMAS JEFFERSON UNIVERSITY
Sidney Kimmel Cancer Center

Interstitial Fluid Pressure Estimation in Breast Cancer using 3D Subharmonic Signals from Contrast-enhanced Ultrasound

Principal Investigator:	Kibo Nam, PhD Department of Radiology 132 South 10th Street, Philadelphia, PA 19107 (215) 955-6261
Co-Investigator(s):	Flemming Forsberg, PhD Department of Radiology 132 South 10th Street, Philadelphia, PA 19107 (215) 955-4870 John Eisenbrey, PhD Department of Radiology 132 South 10th Street, Philadelphia, PA 19107 (215) 503-5188 Andrej Lyshchik, MD, PhD Department of Radiology 132 South 10th Street, Philadelphia, PA 19107 (215) 955-8558 Lydia Liao, MD Department of Radiology 1100 Walnut Street, Philadelphia, PA 19107 (215) 955-9545 Jason Shames, MD Department of Radiology 1100 Walnut Street, Philadelphia, PA 19107 (215) 503-3724 Maysa Abu-Khalaf, MD Department of Medical Oncology 1025 Walnut Street, Suite 700, Philadelphia, PA 19107 (215) 955-8874 Christopher Sebastiano, MD Department of Pathology 132 South 10th Street, Philadelphia, PA 19107 (215) 955-1197
Statistician:	Scott Keith, PhD Pharmacology and Experimental Therapeutics 1015 Chestnut Street, Philadelphia, PA 19107

	(215) 503-9876
Funding Sponsor:	R37 CA234428 National Cancer Institute National Institutes of Health (NIH) 9000 Rockville Pike, Bethesda, MD 20892 (301) 402-9612 Additional Support: Lantheus Medical Imaging
IND Holder:	Flemming Forsberg
IND Number:	IND no. 112,241
Study Product:	Perflutren lipid microspheres; DEFINITY
Protocol IDs:	JeffTrial # 14825 PRC # pending IRB Control # 20G605

Version Number:	**Version Date:**
2.0	2019-08-21
3.0	2019-09-03
3.1	2019-09-23
3.2	2020-03-25
3.3	2020-06-16
4.0	2021-04-20

Table of Contents

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 US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Name: Kibo Nam, PhD

Title: Research Assistant Professor

Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and Thomas Jefferson University research policies

List of Abbreviations

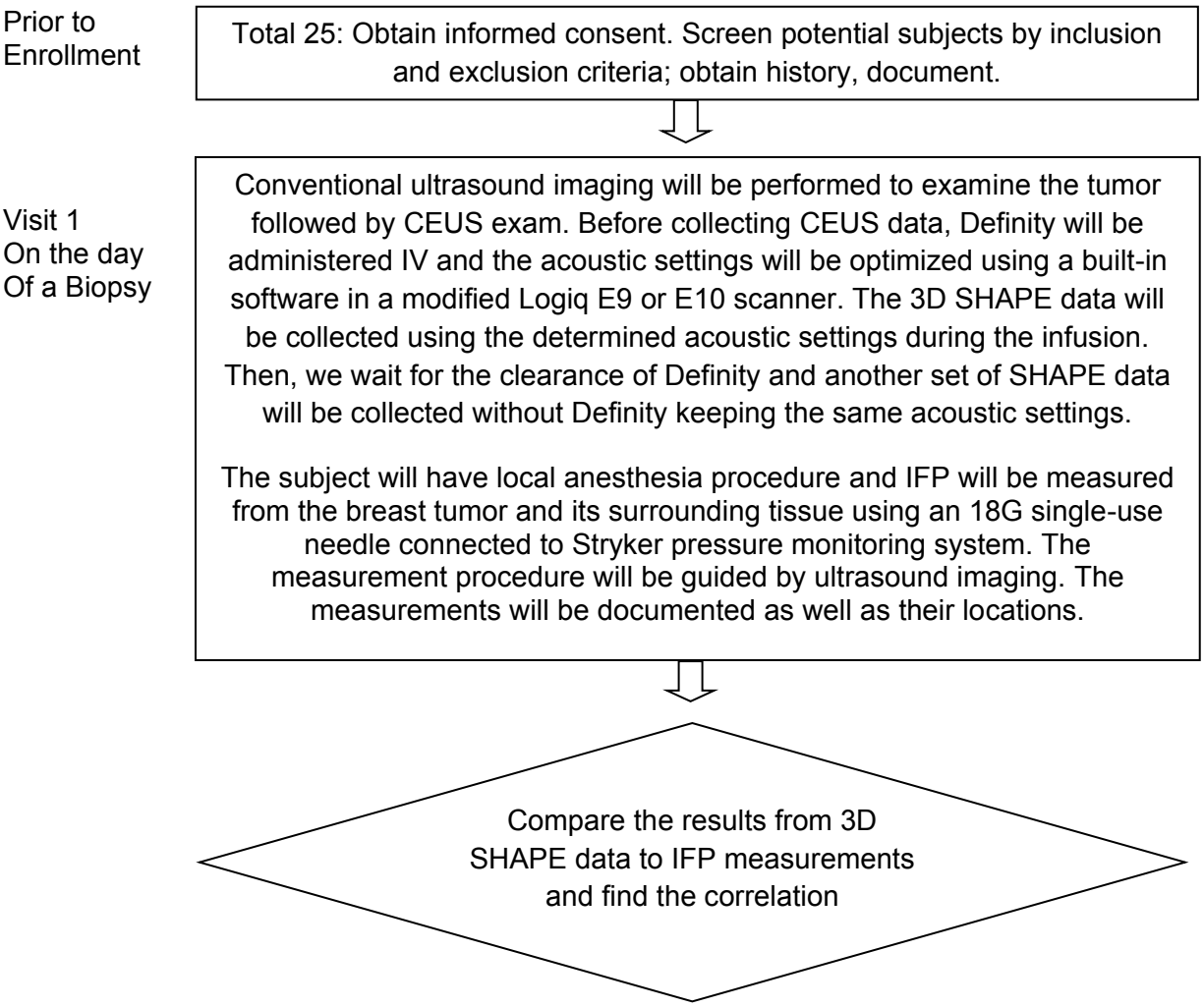
AE	Adverse Event/Adverse Experience
CARPA	Complement Activation Related Pseudo Allergy
CEUS	Contrast-enhanced Ultrasound
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
ICH	International Conference on Harmonisation
IFP	Interstitial Fluid Pressure
IND	Investigational New Drug Application
IRB	Institutional Review Board
LABC	Locally Advanced Breast Cancer
N	Number (typically refers to participants)
NAC	Neoadjuvant Chemotherapy
NCI	National Cancer Institute
NIH	National Institutes of Health
PI	Principal Investigator
PRC	Protocol Review Committee
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDS	Safety Data Sheet (formerly MSDS; Material Safety Data Sheet)
SHAPE	Subharmonic Aided Pressure Estimation
SKCC	Sidney Kimmel Cancer Center
TJU	Thomas Jefferson University
UAP	Unanticipated Problem

Study Summary

Title:	Interstitial Fluid Pressure Estimation in Breast Cancer using 3D Subharmonic Signals from Contrast-enhanced Ultrasound
Précis:	Breast cancer patients scheduled for a breast biopsy will undergo **3D Contrast-Enhanced Ultrasound** (CEUS) exam with a Logiq E9 or E10 scanner (GE Healthcare, Milwaukee, WI) using the ultrasound contrast agent Definity (Lantheus Medical Imaging, Billerica, MA). After CEUS, the measurements of interstitial fluid pressure (IFP) in the tumor and its surrounding tissue (i.e., two sites) using an intra-compartmental pressure monitoring system (Stryker, Newbury, Berkshire, UK) will be performed guided by conventional ultrasound.
Objectives:	**Primary:** To determine if the pressure estimates from 3D Subharmonic Aided Pressure Estimation (SHAPE) technique is correlated with the direct measurements of IFP in breast tumors and the surrounding tissue **Secondary:** To determine if the pressure estimates from 3D SHAPE technique have diagnostic information to detect the malignancy of breast tumors
Population:	The patient population of this study will reflect the demographics of female breast cancer incidence in the city of Philadelphia. The overall demographics include 30% Caucasian, 29% African American, 20% Asian, and 21% Hispanic patients. The sample size of this study was determined to be 25 subjects as a pilot study requiring invasive pressure measurements. The patients enrolled in this project will be female over the age of 21 scheduled for a breast biopsy. The exclusion criteria include patients with known hypersensitivity or allergy to any component of Definity as well as patients with unstable cardiopulmonary conditions or respiratory distress syndrome.
Phase:	Pilot
Number of Sites:	One; Thomas Jefferson University
Description of Intervention:	Patients will first undergo baseline ultrasound imaging. B-mode measurements and sweeps of breast tumor in the transverse and sagittal planes will be acquired. Following baseline imaging, patients will have an administration of ultrasound contrast agent. The ultrasound contrast administration will be an intravenous infusion of 2 vials of Definity/50ml saline will be administered as an IV infusion via a peripheral vein (preferably the antecubital vein), with

	infusion rates of 4 to 10 ml/min (titrated to effect) through an 18-22 gauge angiocatheter. During the contrast infusion, 3D CEUS data will be collected.
Study Duration:	15 months
Participant Participation Duration:	The participation duration is about 80-90 minutes (including 30 minute-long monitoring time) in a single visit.
Estimated Time to Complete Enrollment:	15 months

Schematic of Study Design:



1 Introduction

1.1 Background Information

It has been shown that solid tumors have higher interstitial fluid pressure (IFP) compared to normal tissue. Proliferating tumor cells produce solid stress that compresses blood and lymphatic vessels causing vessel leakiness and lack of functional lymphatics. As a result, IFP becomes significantly elevated in solid tumors [Fukumura et al. 2007, Milosevic et al. 1999]. A study showed that solid tumors had higher IFP compared to normal tissue by 10-25 mmHg using mice with four human tumor cell lines [Stohrer et al. 2000]. Nathanson et al. [1994] measured IFP in the operating room in 25 patients undergoing excision breast biopsy under local anesthetic for diagnostic purposes. They found that invasive ductal carcinomas' mean IFP was 29 mmHg, while those of normal breast tissue, benign tumor, noninvasive carcinomas, and tumor with other benign breast conditions were between -0.3-3.6 mmHg. Additionally, increased IFP prevents an effective uptake of therapeutic agents and reduces the efficacy of cancer therapy [Heldin et al. 2004]. Patients with high IFP had a greater chance to recur after radiotherapy and die of progressive disease than those with low IFP [Milosevic et al. 2001]. Also early decreases in IFP during chemotherapy were highly correlated with late decreases in breast tumor volume in an animal study [Ferretti et al. 2009]. Thus, it can be clinically helpful to measure IFP in breast cancer patients.

1.2 Rationale for the Proposed Study

The IFP of breast tumors can be directly measured invasively using a wick-in-needle (WIN) technique, which employs a catheter connected to a transducer to record the pressures [Taghian et al. 2005]. However, this invasive method is difficult to apply in patients and not standard of care. Contrast-enhanced ultrasound (CEUS) has shown potential to estimate tumor IFP in animals [Halldorsdottir et al. 2014]. CEUS utilizes microbubbles, which not only enhance the backscattered signals, but at higher acoustic pressures also act as nonlinear oscillators producing multiple harmonics of the fundamental frequency. Microbubbles can also act as pressure sensors, due to the difference in compressibility between the bubble and the surrounding medium. Our group has developed the fundamental concept of subharmonic (half of fundamental frequency) aided pressure estimation (SHAPE) using CEUS and demonstrated its potential to non-invasively measure IFP [Halldorsdottir et al. 2014]. The SHAPE method estimates IFP using the inverse relationship between the CEUS signal magnitude at the subharmonic frequency and IFP. This inverse relationship varies with the applied acoustic pressure and an individual optimization of the acoustic setting for SHAPE is therefore needed [Shi et al. 1999]. Recently, we conducted a pilot study of 3D SHAPE's ability to monitor treatment response in women undergoing neoadjuvant chemotherapy (NAC) for locally advanced breast cancer (LABC) [Nam et al. 2017], showing that the subharmonic signal difference in the tumor relative to the normal tissue could predict treatment response after administration of 10% of therapy regimen (i.e., 1 cycle of neoadjuvant regimen). This study did not measure IFP directly, therefore the correlation between IFP and subharmonic signal magnitude has not been studied yet. Thus, in this

study, we will determine if the 3D SHAPE can estimate IFP in breast tumors and the surrounding normal breast tissue noninvasively.

1.3 Correlative Studies

Non-invasively, diffusion-weighted MRI and dynamic contrast-enhanced (DCE) MRI have been suggested as alternative methods for estimating tumor IFP [Hompland et al. 2014]. However, this technique has not been widely adopted in the clinic. Moreover, MRI is expensive and may not be tolerated by all patients, including those with claustrophobia, metal implants, and compromised renal function.

1.4 Potential Risks and Benefits

This study is composed of two parts: Contrast Enhanced Ultrasound (CEUS) with ultrasound contrast agent Definity and invasive Interstitial Fluid Pressure (IFP) measurements using Stryker compartment pressure monitoring system (Kalamazoo, MI).

1.4.1 Potential Risks

Serious cardiopulmonary and allergic reactions including fatalities have occurred during or following administration of Definity. However, these occurrences have been rare (less than 1 in 50,000 patients). As a result, patients with unstable cardiopulmonary conditions will be excluded. The majority of adverse events from Defnity were mild to moderate in severity. Transient side effects that have been described as possibly related to Definity administration include headache (2.3%), back and renal pain (2.1%), flushing (1.1%) and nausea (1.0%). Hypersensitivity reactions to perflutren may occur, although extremely rare.

In a meta-analysis (which included 110,500 patients) [Khawaja et al. 2010] performed early after the FDA safety alerts in 2007, the incidence of serious allergic and anaphylactoid reactions immediately after the ultrasound contrast agent administration was estimated at 0.009% and 0.004%, respectively. These very rare but severe allergic reactions are secondary to a recently described variant of the type1 hypersensitivity reaction known as Complement Activation Related Pseudo Allergy, or CARPA reactions. [Szebeni 2005].

In a retrospective multicenter study, Wei et al [2008] reported on the safety of Definity (n=66164 doses) and Optison (n=12219 doses)- these included >10000 doses administered to critically ill patients in the intensive care unit or to patients with presumed ischemic chest pain. Severe reactions likely attributable to the contrast agents occurred in 8 patients (0.01%), and 4 of these were anaphylactoid reactions. Interestingly, all of the severe reactions occurred in outpatients, indicating that the FDA warning on contrast administration in patients with severe cardiopulmonary conditions likely would not have mitigated risk in these patients.

CARPA reactions may be mild to moderate (sneezing, tingling sensation, urticaria, or pruritis) or severe (wheezing, angioedema, cyanosis, and anaphylactic shock). CARPA

reactions seem more common when a large bolus of lipid shelled contrast agent is injected, and slow continuous infusions of diluted contrast likely afford significant protection against these reactions.

The use of an intravenous needle and the fluids given through the needle may cause minor discomfort, bleeding under the skin (bruise), fainting, and possible infection at the site of needle insertion. These risks are minimized by having nursing staff trained in placing IV lines perform these procedures in a safe, aseptic manner. The subjects' alternative is not to participate in this study and have only their scheduled clinical procedure(s) performed.

The invasive pressure measurement procedure (with 18G needle) has low risk similar to a stereotactic breast biopsy (mostly with 14G or larger needles). The risks includes: bruising and swelling of the breast, infection of the measurement site, and soreness at the needle injection. Also, there is low risk of infection and pain and they could be minimized by strict sterile technique and local lidocaine infiltration, respectively. The subjects' alternative is not to participate in the study.

1.4.2 Benefits

We do not expect any direct benefit for subjects enrolled in this study. The long-term benefits of this study will be the development of a noninvasive, safe, accurate IFP estimator. Additionally, it may lead to a novel functional predictor of malignancy in breast tumors.

2 Study Objectives

2.1 Objectives

This study is designed to compare the direct measurements of IFP in the breast tumor and its surrounding tissue with the values obtained from 3D SHAPE data (i.e., CEUS data).

2.1.1 Primary

The primary objective of the study is to determine if the 3D SHAPE results can be used to estimate IFPs in breast tumor and its surrounding tissue.

2.1.2 Secondary

The secondary objective of the study is to determine if the malignancy of breast tumor can be predicted by the 3D SHAPE results.

2.2 Endpoints/Outcome Measures

2.2.1 Primary

A Pearson's correlation coefficient above 0.5 will be considered a successful outcome.

2.2.2 Secondary

We will use the results from the biopsy as the reference standard. The significance level of Generalized linear mixed logistic or GEE logistic modeling as well as the Mann-Whitney or Student's t-test will be 0.05.

3 Study Design

3.1 Characteristics

This is an open-label, non-randomized, human trial that will be conducted at one clinical site, Thomas Jefferson University. Before IFP measurement, all participants will have an unenhanced (baseline) ultrasound imaging study followed by CEUS imaging study with a single infusion of Definity. Then, they will have the IFP measurements taken by Stryker compartment pressure monitoring system from a breast tumor and its surrounding tissue with ultrasound imaging guide.

3.2 Number of Participants

We plan to enroll 25 participants.

3.3 Duration of Therapy

The total infusion of Definity will be about 15 minutes.

3.4 Duration of Follow Up

All participants will have a 30-minute monitoring period after the administration of Definity.

3.5 Study Timeline

The period of this study is 15 months and the primary and secondary studies will be completed at the same time.

Year 1

(a) Begin enrolling patients (Quarter 1)
(b) Active patient enrollment (Quarter 2-4)
(c) 3D SHAPE data analysis (Quarter 2-4)

Year 2

(a) Active patient enrollment (Quarter 5)
(b) 3D SHAPE data analysis (Quarter 5)
(c) Statistical analysis and preparation for publications (Quarter 5)

3.5.1 Primary Completion

The primary study will completed after 15 months.

3.5.2 Study Completion

The study will completed after 15 months.

4 Study Enrollment and Withdrawal

4.1 Eligibility Criteria

4.1.1 Inclusion Criteria

Individuals must meet all of the following inclusion criteria in order to be eligible to participate in the study:

1. Provide signed and dated informed consent form
2. Be conscious, willing and able to comply with all study procedures and be available for the duration of the study
3. Female, at least 21 years old
4. Be scheduled for a breast biopsy (BIRAD 4, 4A, 4B, 4C or 5)
5. Patient with an at least 1 cm mass located at <3cm depth, approachable by 2.5 inch needle
6. Be medically stable as determined by the investigator
7. If a female of child-bearing potential, must have a negative urine pregnancy test within 24 hours prior to administration of Definity.

4.1.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Males
2. Females who are pregnant or nursing
3. Patients who are medically unstable, patients who are seriously or terminally ill, and patients whose clinical course is unpredictable. For example:
    a. Patients on life support or in a critical care unit.
    b. Patients with unstable occlusive disease (e.g., crescendo angina).
    c. Patients with clinically unstable cardiac arrhythmias, such as recurrent ventricular tachycardia.

d. Patients with uncontrolled congestive heart failure (NYHA Class IV).
e. Patients with recent cerebral hemorrhage.
f. Patients who have undergone surgery within 24 hours prior to the study sonographic examination.

4. Patients with known hypersensitivity or allergy to any component of Definity.
5. Patients with unstable cardiopulmonary conditions or respiratory distress syndrome.
6. Patients with uncontrollable emphysema, pulmonary vasculitis, pulmonary hypertension or a history of pulmonary emboli.

4.2 Gender/Minority/Pediatric Inclusion for Research

We include only female 21 years of age or older regardless of race and speaking language. Since this study does not provide direct benefit to subjects, we determined the age limit of 21 so no state law requires parent's consent for our subject to participate in the study. In 2019, an estimated 268,600 new cases of invasive breast cancer are expected to be diagnosed in women while about 2,670 new cases of invasive breast cancer are expected to be diagnosed in men. The lifetime risk (up to age 95 and older) of breast cancer for women in US is about 13%. However, this risk varies by race and ethnic group: white- 13%, Black- 12%, Asian/Pacific Islander- 11%, Hispanic- 10%, American Indian/Alaska Native- 8%.

4.3 Strategies for Recruitment and Retention

Subjects eligible for trial enrollment will be identified by the investigators from the TJU patient population who are scheduled for a breast biopsy. An investigator or research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The consent form will be reviewed with the patient and then the patient will be given the form to review. If consent interview is conducted by a coordinator, a study investigator will then discuss the study with the subject and answer any additional questions. The patient, person conducting study interview (if applicable), and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for her records.

Screening assessments will be performed within 24 hours prior to the administration of Definity. Trial participants will have the presence of inclusion criteria and absence of exclusion criteria verified by providing a medical history. A full demographic profile, known drug allergies or intolerances, and a review of the subject's medical/surgical history will be recorded. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to the subject prior to study initiation).

4.4 Participant Withdrawal

4.4.1 Reasons for Withdrawal

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study participant's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

4.4.2 Handling of Participant Withdrawals and Participant Discontinuation of Study Intervention

The date the subject is withdrawn from the trial and the reason for discontinuation will be recorded on the CRF. If a subject's participation in the trial is interrupted for any reason (e.g., because of an AE) and the subject has met the criteria described above for completing the trial, the subject's trial participation will be considered completed. If a subject's trial participation is interrupted for any reason by the subject's or investigator's choice and the subject has not met all of the criteria listed above, then the subject will be considered as an incomplete participant.

4.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigators, funding agency, the Investigational New Drug (IND) sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.

5 Study Intervention

5.1 Study Product

This study uses the ultrasound contrast agent, Definity (Lantheus Medical Imaging, Billerica, MA).

5.2 Study Product Description

Definity is a sterile, injectable cardiovascular ultrasound contrast agent comprised of lipid-coated echogenic microbubbles (majority diameter of 1-4 micrometers) filled with octafluoropropane gas and approved by the FDA for use in cardiology.

5.2.1 Acquisition

Definity will be provided by Lantheus, the manufacturer, directly to Thomas Jefferson University.

5.2.2 Formulation, Packaging, and Labeling

Component	Concentration	Chemical Abstracts Service
Glycerin	>10%	56-81-5
Lipid Blend SG896	<1%	N/A
Water	>50%	7732-18-5
Perfluoropropane	<1%	76-19-7
Propylene Glycol	>1%	57-55-6
Sodium Chloride	<1%	7647-14-5
Disodium orthophosphate	<1%	7782-85-6
Sodium phosphate monobasic	<1%	10049-21-5

Definity is supplied as a single use 2 ml clear glass vial containing clear liquid in packages of 4 and 16 single-use vials. It is labeled for the use in patients with suboptimal echocardiograms to opacity the left ventricular chamber and to improve the delineation of the left ventricular endocardial border. In this study, Definity will be used off-label with FDA approval under an IND (no. 112,241).

5.2.3 Product Storage and Stability

Definity needs to be stored between 2-8°C (36°-46°F). The microbubbles are stable and it is necessary to activate them before use.

5.3 Dosage, Preparation, and Administration

Definity will be at room temperature to warm before starting the activation procedure. Definity will be activated by shaking the vial for 45 seconds using the VIALMIX® apparatus (Lantheus Medical Imaging, North Billerica, MA) as suggested by the manufacturer. Two vials of Definity (3 ml in total) suspended in 50 ml saline will be administered as an IV infusion via a peripheral vein (preferably the antecubital vein), with infusion rates of 4 to 10 ml/min (titrated to effect).

5.4 Dose Modifications and Dosing Delays

The recommended infusion dose for the labeled echocardiograms is 1.3 ml and it was modified to 3 ml for this study in accordance with our prior experience in CEUS studies [Sridharan et al. 2013, Nam et al. 2017]. This dose typically provides 5 to 8 minutes of enhancement. Definity is reported to be well tolerated by patients. The largest safety studies published to date on the use of ultrasound contrast agents in humans (involving up to 4,300,966 subjects) concluded that these agents have a good safety profile in both cardiac and abdominal ultrasound applications [Main et al. 2009, Dolan et al. 2009, Wei et al. 2008]. However, as rare but serious cardiopulmonary reactions have been reported following the IV administration of Definity, all subjects will be monitored by a physician during the entire imaging period (as recommended by the FDA).

5.5 Study Product Accountability

The agent will be stored in a secure refrigerator, with only the study investigators and research personnel having access. The study researchers will be responsible for inventory control. The manufacturer will distribute Definity directly to TJU. Any unused product will be disposed at the study site.

5.6 Dietary Restrictions

There is no dietary restrictions for the study.

5.7 Administration of Procedural Intervention

Definity will be administered as an IV infusion via a peripheral vein (preferably the antecubital vein), with infusion rates of 4 to 10 ml/min (titrated to effect) through an 18 to 22 gauge angio-catheter.

5.8 Procedures for Training of Clinicians on Procedural Intervention

The staff in this study are trained for the administration of Definity from our previous studies. If there are clinicians who are not familiar with Definity, we will train them before their participation in the study. If the last training case is found appropriate, the clinical site will be allowed to proceed with the study and training cases will be included in the study analysis. If not, the site personnel will be re-trained.

5.9 Assessment of Clinician and/or Participant Compliance with Study Procedural Intervention

The assessment of clinician and participant compliance with the procedural intervention will be subject to a strict QA review by the study coordinator and investigators at TJU.

6 Study Schedule

6.1 Pretreatment Period/Screening

Patients will be identified and approached from TJU Breast Imaging Center (by investigators). An investigator or research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and to ask questions about participation. A consent form will be reviewed with the patient.

Study participants will have the presence of inclusion criteria and absence of exclusion criteria verified by providing a medical history. A full history and physical examination will be obtained from the patient's referring physician. If the history and physical examination note does not provide adequate information about the patient, the subjects will be assessed for their eligibility by their referring physician before enrollment into the study. If the subject is a woman of childbearing potential, she will have a urine pregnancy test 24 hours prior to the CEUS study (the results of which will be made available to the subject prior to study initiation).

6.2 Enrollment/Baseline

Enrollment/ Visit day (before a biopsy)

- Obtain and document consent from participant on study consent form.
- Verify inclusion/exclusion criteria.
- Obtain demographic information, medical history, and medication history.
- Obtain the previous results of physical and imaging tests if available.
- If woman of childbearing potential, urine pregnancy test within 24 hours prior to receiving Definity (CEUS exam)

6.3 Treatment Period

On the visit day

- Perform Baseline Ultrasound imaging.
- Administer Definity (IV infusion)
    - Determine the optimized acoustic settings for 3D SHAPE imaging
    - Perform CEUS exam and collect 3D SHAPE data

- Wait for clearance of Definity
    - Collect 3D SHAPE data
- Local anesthesia procedures will be performed using simple Lidocaine 1%, or combined with 1:100,000 Epinephrine (EPI) before the IFP measurements.
- Take a single IFP measurement each from the breast tumor and its surrounding tissue using Stryker compartment pressure monitoring system with ultrasound imaging guide

Participants will be monitored for 30 minute following the administration of Definity.

6.4 End of Treatment Study Procedures

We will collect the results from a breast biopsy obtained as standard of care.

6.5 Withdrawal Visit/Discontinuation of Therapy

If participant withdraws early or investigator terminates participant's participation, no additional visits or evaluations will be required or offered to the participant.

7 Study Procedures and Evaluations

7.1 Study Procedures/Evaluations

- Ultrasound, without contrast
- Definity administration
- CEUS exam
- IFP measurements

7.2 Laboratory Procedures/Evaluations

7.2.1 Clinical Laboratory Evaluations

There is no clinical laboratory procedure for this study, except for the urine pregnancy test for women of childbearing potential. We will only collect the results from histology, pathology, and imaging provided as standard of care to evaluate the malignancy of the breast tumor.

8 Evaluation of Safety

8.1.1 Unanticipated Problems

Unanticipated problems (UAPs) include, in general, any incident, experience, or outcome that meets the following criteria:

- 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 participant population being studied;

UAPs are considered to pose risk to participants or others when they suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.2 **Adverse Events**

An adverse event is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research.

8.1.3 **Serious Adverse Events**

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the participant at immediate risk of death from the event as it occurred)
- Is disabling or incapacitating
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant or may require intervention to prevent one of the outcomes listed in this definition.

8.2 **Safety Assessment and Follow-Up**

The subjects will be monitored for AEs and SAEs during the entire procedure and 30 minute monitoring window. All AEs, including observed or volunteered problems, complaints, signs or symptoms, and diagnoses, occurring from the initiation of Definity dosing until the completion of the Definity administration will be recorded on a serious or

non-serious AE data form, whether or not associated with the use of the trial medication.

8.3 Recording Adverse Events

The following subsections detail what information must be documented for each adverse event occurring during the time period specified in Section 8.2 Safety Assessment and Follow-Up.

8.3.1 Relationship to Study Intervention

The relationship to study intervention or study participation must be assessed and documented for all adverse events. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

The following guidelines are used to assess relationship of an event to study intervention:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

8.3.2 Expectedness

The PI or Co-Investigator is responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Risk information to assess expectedness can be obtained from preclinical studies, the investigator's brochure, published medical literature, the protocol, or the informed consent document.

8.3.3 Severity of Event

Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

8.3.4 Intervention

Any intervention implemented to treat the adverse event must be documented for all adverse events.

8.4 Safety Reporting

8.4.1 Reporting to IRB

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section 8.1.1 Unanticipated Problems require the creation and completion of an unanticipated problem report form (OHR-20).

UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 5 working days of the investigator becoming aware of the event.

UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

8.4.1.1 *Adverse Events*

Grade 1 AEs will be reported to the IRB at continuing review.

Grade 2 AEs will be reported to the IRB at the time of continuing review.

8.4.1.2 *Serious Adverse Events*

SAEs will be reported to the IRB on OHR-10 forms via the electronic reporting system (eSAEy) according to the required time frames described below.

Grade 3-4 AEs that are unexpected and deemed to be at least possibly related to the study will be reported to the IRB within 2 working days of knowledge of the event.

Grade 3-4 AEs that are deemed unrelated to the study will be reported to the IRB within 5 working days.

Grade 5 AEs will be reported to the IRB within one working day of knowledge of the event.

All SAEs will be submitted to the IRB at continuing review, including those that were reported previously.

8.4.2 Reporting to SKCC DSMC

All AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.

For expedited reporting requirements, see table below:
DSMC AE/SAE Reporting Requirements

	Grade 1	Grade 2		Grade 3				Grades 4 and 5
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I - 48 Hours (Death: 24 Hours) Phase II - 5 working days
Possible Probably Definite	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	48 Hours (Death: 24 Hours)	Phase I - 48 Hours Phase II - 5 working days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I and Phase II - 48 Hours (Death: 24 Hours)

8.4.3 Reporting to Funding Sponsor

Serious adverse events that occur with commercially available agents/devices are reported through Food and Drug Administration. The manufacturer (Lantheus) will also receive reports.

8.4.4 Reporting to FDA

The investigators will report in an IND safety report any suspected adverse reaction to study treatment (i.e., including active comparators) that is both serious and unexpected (21 CFR 312.32(c)(1)(i)) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. Before submitting an IND safety report, the investigators needs to ensure that the event meets all three of the definitions:

- Suspected adverse reaction
- Serious
- Unexpected

SAEs will be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.
MedWatch SAE forms will be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

If the adverse event does not meet all three of the definitions, it will not be submitted as an IND safety report.

In addition, the investigators will identify in each IND safety report all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and will analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information (21 CFR 312.32(c)(1)). The analysis will include similar reports from all INDs held by the sponsor and any other relevant information known to the sponsor (21 CFR 312.32(c)(1)). Investigators will evaluate a suspected adverse reaction in the context of other related reports or adverse events, including those that occurred in the placebo or active comparator group and those that occurred in pre- and postmarketing studies.

8.4.5 Reporting of Pregnancy

If the subject is a woman of childbearing potential, she will have a urine pregnancy test prior to each CEUS study.

8.5 Halting Rules

Should the observed rate of AE/SAE at any of the scheduled interim analyses exceed 25%, study enrollment may be suspended so that the study committee may consider whether a change in protocol is necessary.

9 Study Oversight

In addition to the PI's responsibility for oversight, study oversight will be under the direction of the SKCC's Data and Safety Monitoring Committee (DSMC). The SKCC DSMC operates in compliance with a Data and Safety Monitoring Plan (DSMP) that is approved by the NCI.

10 Clinical Site Monitoring and Auditing

Clinical site monitoring and auditing is conducted to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring and auditing for this study will be performed in accordance with the SKCC's Data and Safety Monitoring Plan (DSMP) developed by the SKCC Data and Safety Monitoring Committee (DSMC). The DSMP specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of participant data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study site(s). Appropriate staff will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SKCC DSMP.

11 Statistical Considerations

11.1 Study Hypotheses

The main hypothesis is that the 3D subharmonic signals in the tumor and its surrounding tissue are correlated with the IFP values in them. The secondary hypothesis is that a marker from 3D subharmonic signals can predict the malignancy of breast tumor.

11.2 Analysis Plans

Radio frequency (RF) data from 3D SHAPE will be transferred to a PC for off-line analysis. 3D RF data from SHAPE will be composed of multiple 3D volumes (5 to 21 volumes) over time. Each 3D volume will consist of multiple 2D data sets (about 79 slices), which will be collected in the elevational direction. All data processing will be

performed using Matlab (R2015b; Mathworks, Natick, MA). Two regions of interest (ROI) will be selected in each elevational plane for SHAPE: one within the tumor and the other in the surrounding tissues (available top and/or bottom region of tumor). The ROIs of the tumor and surrounding area will be selected as a free form on the images generated from RF data (i.e., raw data before post-processing for on-screen display). The ROIs will be selected by the sonographer or radiologist. Then, a Fast Fourier Transform will be computed for each A-line within the ROI to obtain the frequency spectrum. The maximum magnitude from the resultant frequency spectrum will be extracted from each pre- and during infusion data sets and the ratio will be calculated for each ROI. If both top and bottom regions are selected as a surrounding area ROI, then the ratios will be averaged to one value. Finally, the obtained subharmonic signal ratios from the tumor and the surrounding area will be compared to the direct IFP measurements. Pearson's correlation coefficients will be computed between SHAPE results and direct IFP measurements by Stryker compartment pressure monitoring system. These data will also be rigorously analyzed for agreement using Bland-Altman analysis (Bland & Altman, 1999) among tumor and healthy tissue samples. To explore if the relationship between SHAPE results and IFP is confounded by or might depend on study variables collected (e.g.'s, patient characteristics, tissue/tumor characteristics, etc.) linear mixed effects or generalized estimating equations modeling will be used. Generalized linear mixed logistic or GEE logistic modeling of tumor vs. non-tumor samples will be used to explore the potential for using SHAPE results as a novel functional predictor of breast tumor malignancy. Additionally, the subharmonic signal ratio from the tumor will be used to predict the malignancy of breast tumor using a biopsy result as a gold standard. The Mann-Whitney or Student's t-test will be performed depending on the distribution of the results.

11.3 Sample Size Considerations

The aim of the study is to determine the correlation between SHAPE results and direct measurements of IFP. The aim is to get significant result ($p < 0.05$) with sufficient power (80%) to detect at least correlation coefficient of 0.5-0.6. Therefore, the required sample size for this study is 19 -29 based on the formula for calculation is based on two-tailed test [Guenther 1977]. Thus, we chose 25 patients as our sample size.

11.3.1 Replacement Policy

The patients who consent for the study participation but do not undergo the CEUS exam or IFP measurements will be replaced. Also, unsuccessful training cases will be replaced.

11.3.2 Accrual Estimates

At the Jefferson-Honickman Breast Imaging Center, a breast biopsy is performed on more than 500 women each year. We expect that 25 subjects can be enrolled within 15 months.

12 Source Documents and Access to Source Data/Documents

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, and regulatory and institutional requirements for the protection of confidentiality of participant information. Study staff will permit authorized representatives of SKCC and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

13 Quality Control and Quality Assurance

3D SHAPE data including ultrasound imaging and CRFs will be subject to a strict QA review by the study coordinator and PI at TJU. Results of the QA review will be shared on the quarterly basis with additional training provided, if necessary.

14 Ethics/Protection of Human Participants

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

14.2 Institutional Review Board

The protocol and informed consent form will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be IRB-approved, and the participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be affected if they decline to participate in this study. The consent process will be documented in the clinical or research record. For non-English speaking participants, a translator fluent in both languages, will present and explain the study and full consent form at the time of the consent discussion. In addition, an IRB approved short consent form, translated in the subject's language will be provided to the subject. All parties will sign the translated consent form.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

The subject population of this study will be 100% women over 21 years old. Children, and pregnant women will be excluded based on the benefit and risk assessment. No patient will be excluded based on race.

14.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study participants. The clinical study site will permit access to such records.

14.6 Future Use of Stored Specimens and Other Identifiable Data

No specimens or other identifiable data will be stored for future use.

15 Data Handling and Record Keeping

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents must be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation.

15.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

15.2 Data Capture Methods

All patient data will be captured electronically via excel file in a password protected computer and image and 3D SHAPE data will be stored in the secured computer hard drive. Image and 3D SHAPE data will be de-identified. Copies of study documents will have personal identifying information retracted and replaced with study patient ID number. Originals of the study documents with personal identifying information will be retained at TJU. All this information will be available for audit. In addition, de-identified study data may be shared with study co-sponsors GE Healthcare and Lantheus Medical Imaging.

15.3 Types of Data

The following data will be collected:

- Results of clinical examination before the enrollment
- Breast biopsy schedule and results
- De-identified 3D SHAPE (RF) data, Stryker IFP measurements, and B-mode images from the study

15.4 Study Records Retention

Study records will be maintained for at least three years from the date that the grant federal financial report (FFR) is submitted to the NIH.

Study documents must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the participant, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study participant source documents and promptly reported to the IRB and other regulatory bodies according to their requirements.

16 Study Finances

16.1 Funding Source

This study will be financed through a grant from the US National Institute of Health.

16.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and

approved by the study sponsor prior to participation in this study. All Jefferson University Investigators will follow the TJU Conflicts of Interest Policy for Employees (107.03).

16.3 Participant Stipends or Payments

At the completion of the study (a single visit for CEUS and IFP measurements), the participants will receive a preloaded “clincard” for the amount of $100.

17 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801 mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials:"

Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;

NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

18 Literature References

Bland JM, Altman DG (1999). "Measuring agreement in method comparison studies". Statistical Methods in Medical Research. 8 (2): 135–60.

Ferretti S, Allegrini PR, Becquet MM, McSheehy PM. Tumor interstitial fluid pressure as an early-response marker for anticancer therapeutics. Neoplasia 2009; 11:874-881.

Fukumura D, Jain RK. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. J Cell Biochem 2007; 101:937-949.

Guenther WC. Desk calculation of probabilities for the distribution of the sample correlation coefficient. The American Statistician 1977; 31:45-58.

Halldorsdottir VG, Dave JK, Eisenbrey JR, et al. Subharmonic aided pressure estimation for monitoring interstitial fluid pressure in tumours –in vitro and in vivo proof of concept. Ultrasonics 2014; 54:1938-1944.

Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure-an obstacle in cancer therapy. Nat Rev Cancer 2004; 4:806-813.

Khawaja OA, Shaikh KA, Al-Mallah MH. Meta-analysis of adverse cardiovascular events associated with echocardiographic contrast agents. AmJ Cardiol 2010;106:742–747.

Milosevic MF, Fyles AW, Hill RP. The relationship between elevated interstitial fluid pressure and blood flow in tumors: a bioengineering analysis. Int J Radiat Oncol Biol Phys 1999; 43:1111-11239.

Milosevic M, Fyles A, Hedley D, et al. Interstitial fluid pressure predicts survival in patients with cervix cancer independent of clinical prognostic factors and tumor oxygen measurements. Cancer Res 2001; 61:6400-6405.

Nam K, Eisenbrey JR, Stanczak, et al. Monitoring neoadjuvant chemotherapy for breast cancer by using three-dimensional subharmonic aided pressure estimation and imaging with US contrast agents: preliminary experience. Radiology 2017; 285:53-62.

Nathanson SD, Nelson L. Interstitial fluid pressure in breast cancer, benign breast conditions, and breast parenchyma. Ann Surg Oncol. 1994 Jul; 1:333-338.

Shi WT, Forsberg F, Raichlen JS, Needleman L, Goldberg BB. Pressure dependence of subharmonic signals from contrast microbubbles. Ultrasound Med Biol 1999; 25:275-283.

Sridharan A, Eisenbrey JR, Liu JB, et al. Perfusion estimation using contrast enhanced three-dimensional subharmonic ultrasound imaging: an in vivo study. Invest Radiol 2013; 48:654-660.

Stohrer M, Boucher Y, Stangassinger M, Jain RK. Oncotic pressure in solid tumors is elevated. Cancer Res 2000; 60:4251-4255.

Szebeni J. Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity. Toxicology. 2005;216:106–121.

Taghian AG, Abi-Raad R, Assaad SI, et al. Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancers in patients treated with neoadjuvant chemotherapy: clinical implications. J Clin Oncol 2005; 23:1951-1961.

Wei K, Mulvagh SL, Carson L, et al. The safety of deFinity and Optison for ultrasound image enhancement: a retrospective analysis of 78,383 administered contrast doses. J Am Soc Echocardiogr. 2008;21:1202–1206.

SUPPLEMENTAL MATERIALS

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

- Case Report Forms (CRFs)

Appendices

The following documents are officially affiliated with the protocol and will be submitted to the IRB as a part of the protocol. As such, changes to these items require a protocol amendment.

Appendix A: Schedule of Events

APPENDIX A: SCHEDULE OF EVENTS

Procedures	Screening	Study Visit
Signed Consent Form	X	
Assessment of Eligibility Criteria	X	X
Review of Medical History	X	
Definity Administration		X
CEUS exam		X
Ultrasound without contrast		X
Assessment of Adverse Events		X
IFP Measurements		X
Pregnancy test (for women of childbearing potential)[1]	X	X

1. Urine pregnancy test within 24 hours prior to administration of Definity