LCCC 1745: ARFI, VisR, and DDAI Ultrasound for Improving Discrimination of Malignant and Unresponsive Breast Cancer

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LCCC 1745: ARFI, VisR AND DDAI ULTRASOUND FOR IMPROVING DISCRIMINATION OF MALIGNANT AND UNRESPONSIVE BREAST CANCER

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

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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:_____Caterina Gallippi, Ph.D._____

PI Signature: Caterina M Gallippi

Date:_____12/7/19_____

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

The primary objective of the proposed research is to evaluate *in vivo* the diagnostic relevance of ultrasound-derived metrics for stiffness, elasticity, viscosity, and anisotropy. These biomarkers will be measured using novel, noninvasive ultrasound technologies under development in Dr. Gallippi's laboratory: 1) Acoustic Radiation Force Impulse (ARFI) ultrasound for interrogating tissue stiffness, 2) Viscoelastic Response (VisR) ultrasound for assessing tissue elasticity and viscosity, and 3) Dynamic Displacement Anisotropy Imaging (DDAI) for measuring tissue anisotropy.

We hypothesize that ultrasound-derived stiffness, elasticity, viscosity, and anisotropy will detect lesion malignancy and predict response to treatment. To test this hypothesis, we will pursue the following specific aims:

Aim #1: Quantify the ability of ultrasound-derived stiffness, elasticity, viscosity, and anisotropy to detect malignancy. ARFI, VisR, and DDAI imaging will be performed on suspicious breast lesions in 40 women with BIRADS-4a, 4b, 4c, or -5 ratings. The diagnostic accuracy of imaging metrics will analyzed, with malignancy confirmed by histology as the outcome.

Aim #2: Quantify the ability of ultrasound-derived stiffness, elasticity, viscosity, and anisotropy to predict a positive response to treatment. ARFI, VisR, and DDAI imaging will be performed serially over the course of neoadjuvant chemotherapy (NAC) - on malignant breast lesions in 40 women. Changes in outcome metrics over time will be correlated to overall reduction in tumor size (diameter and area). The ability of ultrasound metrics to predict a positive response to treatment will be examined. A positive response to treatment will be determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.[1,2]

1.2 Disease Background (if applicable)

The primary objective of breast cancer screening is to identify early stage cancer, or precancerous lesions, at a time before symptoms emerge and when treatment is likely to result in a cure. Screening is beneficial when it averts progression of disease to metastasis and/or death, but adverse effects to patients (and unnecessary medical expense) may result downstream from false positives and indiscrimination of masses that will not respond to treatment. The sensitivity of digital mammography, the current screening standard in the US, has been reported in the range of 0.40 to 0.85,[1] with a positive predictive value of 0.31.[2] Sensitivity is increased by augmenting mammography with MRI and B-Mode ultrasound, but false positive rates may also increase[3]. There exists a vital need for a screening technology that exhibits high sensitivity and specificity for cancer detection with early identification of unresponsive masses.[4]

This urgent need could be met by exploiting new imaging biomarkers. Specifically, the mechanical properties of breast tissue have been used for cancer detection, with both elasticity[5–8] and viscosity[9] demonstrated for discriminating malignant from benign lesions. Further, tissue anisotropy has been shown to correlate with core biopsy result and tumor grade, with large cancers significantly more anisotropic than small cancers.[10] Importantly, while both MRI and ultrasound can be used to measure these biomarkers, ultrasound's cost effectiveness and ease of implementation render it an efficient platform to pursue.

1.3 Diagnostic and Prognostic Studies

As stated above, *we hypothesize that ultrasound-derived tissue stiffness, elasticity, viscosity, and anisotropy will detect lesion malignancy and predict response to treatment.* Tissue property measures will be made using Acoustic Radiation Force Impulse (ARFI), Viscoelastic Response (VisR), and Dynamic Displacement Anisotropy Imaging (DDAI), which are described below:

Acoustic Radiation Force Impulse (ARFI) Imaging for Assessing Tissue Stiffness: In ARFI imaging, the ultrasound transducer delivers an impulsive ARF excitation to the tissue near the position of the imaging focus, and, using the same transducer, the induced tissue displacement is monitored over time. Then, parametric images of ARFI-induced peak displacement (PD) are rendered to convey information regarding the relative stiffness of tissue, i.e. stiffer tissues achieve PDs that are smaller than those of softer tissues in response to a consistent ARF magnitude.[11–14] ARFI PD has been used by our group to characterize atherosclerotic plaques and delineate necrotic cores and fibrous caps. Fibrous cap thickness measurement ≥ 0.5 mm has been demonstrated to be within 10% error of histologically derived measurements. This, and previous work by others,[15–21] suggest that ARFI will be similarly relevant for delineating stiffness differences in the breast that will be relevant for detecting necrotic cores, which could aid in discriminating malignant lesions and masses that do not respond to treatment.

Viscoelastic Response (VisR) Ultrasound for Assessing Tissue Elasticity and Viscosity: Unlike ARFI, which primarily assesses tissue stiffness, VisR uses successive ARF excitations to interrogate tissue viscoelasticity.[22–24] ViSR has been used to study muscle degeneration in boys with Duchenne Muscle Dystrophy. The ViSR measurements provided information on the changes in viscosity and elasticity of these muscles and were concordant with inflections in matched physical and quantitative muscle testing (QMT) results. The VisR results are also consistent with expected cycles of dystrophic degeneration. These data support that VisR will similarly be relevant to assessing viscous and elastic properties in the breast, which have been shown to be relevant to discriminating malignant from benign breast lesions[5,6,8,9,25–27] and may be useful for distinguishing responsive from unresponsive masses.

Dynamic Displacement Anisotropy Imaging (DDAI) for Measuring Tissue Anisotropy: Anisotropic tissue exhibits mechanical properties that are directionally dependent, i.e. the viscous and/or elastic properties of the material vary between one direction and another, and the degree of difference is referred to as the "degree of anisotropy."[28] Anisotropy is interrogated in DDAI imaging using ARF that is configured to have an asymmetrical shape.[29] For example, in a transversely isotropic (TI) material, ARFI-induced PDs and VisR τ values are larger when the long axis of the asymmetrical ARF is aligned along versus across the material's axis of symmetry (AoS). The ratio of induced PDs or τ values along versus across the AoS reflects the degree of anisotropy in the material. We have developed DDAI imaging using finite element method (FEM) simulations. These data suggest that DDAI imaging will differentiate breast lesions with anisotropic properties and/or AoS orientations that differ from those of the background breast tissue. Previous work suggests that DDAI-derived anisotropy information will correlate with breast core biopsy result, tumor grade, and tumor size[10,30] and may be useful for differentiating malignant from benign lesions and responsive from unresponsive masses.

We will correlate ultrasound-derived stiffness, elasticity, viscosity, and anisotropy with response to treatment (Aim #2). Please see data analysis plans below for more information.

1.4 Rationale

In this exploratory clinical study, we will attempt to demonstrate that ARFI, VisR, and DDAI ultrasound reliably detect malignant breast masses (Aim #1) and distinguish masses that respond to chemotherapy from those that do not (Aim #2). The ARFI, VisR, and DDAI imaging location will be on the surface of the breast, above the suspicious or malignant mass. This unblinded, open-label, exploratory study will be conducted in 40 women with diagnosed BIRADS breast assessment categories of -4a, 4b, 4c, or -5 masses in Aim #1 and in 40 women with malignant masses undergoing neoadjuvant chemotherapy (NAC) in Aim #2. In Aim #1, patients will be recruited from those who have undergone breast imaging and are indicated for biopsy according to the routine standard of care at UNC Hospitals. Both diagnostic imaging and biopsy will be performed according to institutional standards and will not be altered for research purposes. In addition to standard radiologic examinations, the patients will undergo ARFI, VisR, and DDAI ultrasound imaging after BIRADS diagnosis but prior to biopsy. In Aim #2, patients will be recruited from those already undergoing NAC for treatment of breast cancer according to the standard of care at UNC Hospitals. NAC will be performed based on institutional standards and will not be altered for research purposes, except that we will perform serial ultrasound imaging over the course of treatment. The standard for clinical management during and after NAC will not be altered.

We will enroll participants in two groups, as patients with suspicious (Aim #1) breast lesions, or as patients with confirmed breast cancer undergoing clinically

indicated NAC (Aim #2). There will be no randomization to study arms and no intervention beyond ARFI, VisR, and DDAI ultrasound imaging.

1.5 Compliance Statement

This study will be conducted in full accordance to all applicable University of North Carolina (UNC) Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unexpected problems in accordance with The UNC IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2.0 STUDY OBJECTIVES

The purpose of this study is to evaluate in vivo the diagnostic relevance of ultrasound-derived stiffness, elasticity, viscosity, and anisotropy metrics for breast lesion malignancy and response to treatment.

2.1 Aim #1: Quantify the ability of ultrasound-derived stiffness, elasticity, viscosity, and anisotropy to detect malignancy.

In vivo ARFI, VisR, and DDAI imaging will be performed in 40 women with a breast mass with a BIRADS breast assessment categories of -4a, 4b, 4c, or -5 rating after diagnostic workup. First, the transducer will be placed lightly above the suspicious mass, just to the point of skin contact, to avoid pre-compression during imaging.[15] The suspicious mass will be positioned at 10 to 40 mm in axial depth, with standoff used if needed to achieve the desired axial position. Second, the position of the transducer will be rotated through 90^{o} in steps of 30^{o} to identify the transducer position yielding maximum ARFI peak displacement in the mass. The transducer will be stabilized in this position, with light skin contact, by the sonographer or by using a stereotactic clamp. Third, ARFI and VisR data will be acquired in immediate succession. Fourth, the transducer will be rotated 90^{o}, re-stabilized by the sonographer or by using the stereotactic clamp, and ARFI and VisR data will be acquired in the new transducer position to enable DDAI analysis. Finally, the evaluated mass will undergo clinically indicated biopsy with histological evaluation to determine if it is benign or malignant.

The ultrasound data will be processed to calculate the following outcome metrics: peak displacement (PD), displacement at a given time (TD) for ARFI; τ, relative elasticity (RE), relative viscosity (RV), and tissue mass (TM) for VisR; ratio of ARFI PD and ratio of VisR RE values at 90^{o} versus 0^{o} transducer orientations for DDAI. These eight outcome metrics, alone and in combinations, will be statistically correlated with biopsy finding of malignant or benign.

2.1.1 Determine which of the 8 ultrasound metrics best detects malignancy.

We will statistically evaluate all eight ultrasound outcome metrics independently to determine which metric best detects malignant finding.

2.1.2 Determine the combined diagnostic accuracy of any 2 ultrasound metrics.

We will systematically combine all possible groupings of two ultrasound outcome metrics. We will then determine which grouping of metrics best detects malignant finding.

2.1.3 Determine the combined diagnostic accuracy of ultrasound metrics and mammography.

For ultrasound outcome metrics (singly or combined) with AUC exceeding 0.75 in 2.1.1 and 2.1.2, we will determine if combining the metrics with mammography-derived mass size, density, shape, and margins improves ROC performance relative to not combining with mammography.

2.1.4 Determine which cutpoints for ultrasound metrics detect malignancy with optimum sensitivity and specificity.

Best-balanced cutpoints from the highest performing ultrasound metrics in 2.1.1 will be derived, and PPV will be calculated.

2.2 Aim #2: Quantify the ability of ultrasound-derived stiffness, elasticity, viscosity, and anisotropy to predict a positive response to treatment.

In 40 women with newly diagnosed invasive breast cancer who are scheduled to begin clinically indicated NAC, ARFI, VisR, and DDAI imaging will be performed (as described in Aim #1) immediately before beginning treatment and then over the course of treatment. The eight ARFI, VisR, and DDAI outcome metrics described in Aim #1 will be statistically correlated with clinically determined change in tumor size following NAC treatment. ARFI, VisR, and DDAI outcome metrics will also be evaluated for ability to predict positive response to NAC, with positive response determined according to the RECIST guidelines.[1,2]

2.2.1 Determine which baseline ultrasound metric best predicts positive response to NAC.

We will statistically determine, from among all eight ultrasound outcome metrics acquired at each imaging time point, which metric best predicts positive response to treatment, with positive response determined according to the RECIST guidelines.[1,2]

2.2.2 Determine which baseline ultrasound metric correlates most strongly with absolute reduction in tumor size.

We will correlate absolute change in tumor size (diameter and area) from baseline to end of chemotherapy type with ultrasound metric values at baseline.

2.2.3 Determine the combined ability of any 2 baseline ultrasound metrics for prediction of positive response to NAC.

We will systematically combine all possible groupings of any two ultrasound outcome metric values at baseline. We will then statistically analyze the combined ability to predict reduction in tumor size.

2.2.4 Determine if serial changes in ultrasound metrics correlate with positive response to NAC.

We will evaluate the correlation of serial changes in ultrasound metrics with tumor size. Assessments of serial change will be made at 3 intervals per chemotherapy type: baseline, early chemotherapy type treatment, and end of chemotherapy type treatment.

2.2.5 Determine for which of the 8 ultrasound metrics do serial changes best predict positive response to NAC.

The predictive ability of serial change in each ultrasound metric from baseline to end of chemotherapy type will be analyzed statistically. We will systematically combine all possible groupings of serial changes in any two ultrasound outcome metrics. We will then statistically analyze the combined ability to predict positive response to NAC.

2.2.6 Determine if combinations of changes over time in any ultrasound outcome metrics correlate with positive response to NAC.

We will systematically combine all possible groupings of changes over time in any two ultrasound outcome metrics. We will then statistically analyze the combined ability to predict positive response to NAC.

2.2.7 Determine if combinations of serial change for any 2 ultrasound metrics predict positive response to NAC.

We will assess the predictive ability of serial change in ultrasound metrics from baseline to end of chemotherapy type treatment using ROC analysis.

2.2.8 Determine the combined performance of ultrasound metrics and mammography for predicting a positive response to NAC.

For ultrasound outcome metrics with an AUC exceeding 0.75 from 2.2.1, 2.2.3, 2.2.5, and 2.2.7, their combined diagnostic accuracy with mammography measures (mass size, density, shape, and margins) will be analyzed statistically.

2.2.9 Determine the sensitivity, specificity and PPV of ultrasound outcome metrics to predict positive response to NAC.

Best-balanced cutpoints from the highest performing ultrasound metrics in 2.2.1 and 2.2.4 will be derived used to analyze sensitivity, specificity, and PPV of reduction in tumor size.

2.3 Endpoints

Primary endpoints are ARFI PD and TD, VisR τ, RE, RV and RM, and DDAI degree of anisotropy measured as the ratio of PD and as the ratio of RE values. The secondary endpoints are changes in these eight ultrasound-derived metrics over time. These primary and secondary endpoints will be used for Aims #1 and #2 objectives listed above.

3.0 PATIENT ELIGIBILITY

In Aim #1, patients will be recruited from those who have undergone breast imaging and are indicated for biopsy according to the routine standard of care at UNC Hospitals. Both diagnostic imaging and biopsy will be performed according to institutional standards and will not be altered for research purposes. In addition to standard radiologic examinations, the patients will undergo ARFI, VisR, and DDAI ultrasound imaging prior to standard of care biopsy..

In Aim #2, patients will be recruited from those already undergoing NAC for treatment of breast cancer according to the standard of care at UNC Hospitals. NAC will be performed based on institutional standards and will not be altered for research purposes, except that we will perform serial ultrasound imaging over the course of treatment. The standard for clinical management during and after NAC will not be altered.

Potential study participants will be patients 30-90 years of age who have been diagnosed by their treating physician to have a BIRADS breast assessment categories of -4a, 4b, 4c, or -5 breast lesion (Aim #1) or to be in need of NAC for the treatment of breast cancer (Aim #2). All patients will be recruited from and imaged at the University of North Carolina Hospitals. Based on local demographics, we estimate that roughly 20% will be racial and ethnic minorities. Children are not likely to have breast cancer and will not be included in this study.

We will enroll participants in two groups, as patients with suspicious (Aim #1) breast lesions, or as patients with confirmed breast cancer undergoing clinically indicated NAC (Aim #2). There will be no randomization to study arms and no intervention beyond ARFI, VisR, and DDAI ultrasound imaging.

3.1 Inclusion Criteria

Subjects must meet all of the inclusion criteria for either Aim 1 or Aim 2 in order to participate in this study.

3.1.1 Aim 1

3.1.1.1 Patients are aged 30-90 years

3.1.1.2 Patients with BIRADS 4a, 4b, 4c, or 5 rating

3.1.1.3 Lesion is sonographically visible with B-Mode ultrasound on diagnostic workup

3.1.1.4 Informed consent obtained and signed

3.1.2 Aim 2

3.1.2.1 Patients are aged 30-90 years

3.1.2.2 Patients who are or will be undergoing neoadjuvant chemotherapy (NAC) for TNM staging of T1-T4, N0-N3 and M0-M1

3.1.2.3 Lesion is sonographically visible with B-Mode ultrasound on diagnostic workup

3.1.2.4 Informed consent obtained and signed

3.2 Exclusion Criteria

Subjects who meet any of the exclusion criteria will be excluded from study participation for both Aim 1 and Aim 2. (With the exception for Aim 2 – 3.2.5, since those subjects would have had a biopsy-proven malignant lesion).

3.2.1 Inability to provide informed consent

3.2.2 Inability to communicate in English

3.2.3 Inability to remain motionless for 15 minutes

3.2.4 Suspicious or malignant breast mass deeper than 3 cm from skin surface

3.2.5 Aim 1 - Previous biopsy or surgery to the site of the suspicious or malignant mass. Aim 2 – Surgical excision of biopsy-proven lesion of interest.

3.2.6 Patients who are pregnant

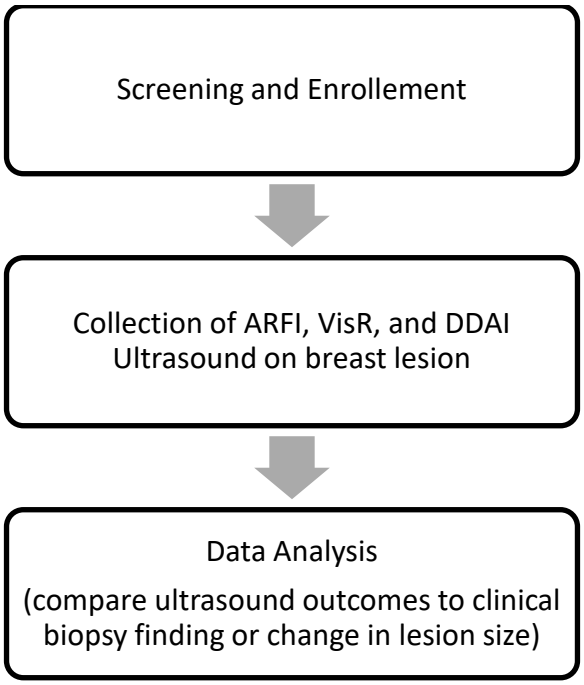
3.2.7 Patients who are lactating

3.2.8 Patients with a history of mastectomy

3.2.9 Patients with breast implants

4.0 STUDY PLAN

4.1 Schema



4.2 Imaging Schedule

Study enrollment will occur for approximately 18 months, with anticipated recruitment of 4-5 patients per month. Total ultrasonic imaging time for all imaging conditions (ARFI, VisR, DDAI) is approximately 15 minutes. Therefore, Subjects participating in Aim 1 of the study will participate for an expected duration of 15 minutes. Subjects participating in Aim 2 will be participating for an expected duration of approximately 15 minutes per imaging session during the duration of their NAC treatment as outlined in Section 4.2.2.

4.2.1 Arm 1

In addition to standard radiologic examinations, the patients will undergo ARFI, VisR, and DDAI ultrasound imaging after BIRADS diagnosis but prior to biopsy.

4.2.2 Arm 2

Patients in Arm 2 will be imaged to align with their clinical treatment schedule. Imaging will occur at baseline, at 2 – 3 weeks (+/- 7 days) into therapy (driven by treatment plan), and at completion of each chemotherapy type. In some cases, the final US of one chemotherapy type will also serve as the baseline for the next regimen. The below schedule has been included for clarity and as an example. Patients undergoing any NAC regimen will be included and their imaging will align with their treatment at baseline, early treatment by chemotherapy type, and the end of each chemotherapy type. In the event that subjects undergo more than 3 rounds of chemotherapy, an additional scan will be completed at the end of all chemotherapy. At minimum, patients would undergo 3 US scans, and at maximum of 8 US scans.

	Baseline Chemotherapy Type 1	**Early Chemotherapy Type 1**	**Completion of Chemotherapy Type 1/ Baseline Chemotherapy Type 2**	**Early Chemotherapy Type 2**	**Completion of Chemotherapy Type 2/ Baseline Chemotherapy Type 3**	**Early Chemotherapy Type 3**	**Completion of Chemotherapy Type 3**
ER+/HER2-	day 0	day 14 (+/-7 days)	end of chemotherapy type (+/- 7 days)	day 14 (+/-7 days)	end of chemotherapy type (+/- 7 days)	day 14 (+/-7 days)	end of chemotherapy type (+/- 7 days)
TNBC	day 0	day 14 (+/-7 days)	end of chemotherapy type (+/- 7 days)	day 21 (+/-7 days)	end of chemotherapy type (+/- 7 days)	day 14 (+/-7 days)	end of chemotherapy type (+/- 7 days)
HER2+	day 0	day 14 or 21 (+/-7 days) depending on treatment schedule	end of chemotherapy type (+/- 7 days)	day 21 (+/-7 days)	end of chemotherapy type (+/- 7 days)	N/A	N/A

4.3 Patient Identification and Consent

Informed consent will occur prior to participants receiving any sedation for biopsy or NAC, should it be needed. The informed consent will be a written document explaining procedures and risks of the study. The Investigator will review the consent form with the subject and will answer any questions potential participants might have. Once all questions have been answered, the subject will be asked if they still want to participate, and if so, to sign the consent and HIPAA documents.

4.4 Abstraction of Medical Records (if applicable)

Patient's medical record will be reviewed to assess response to neoadjuvant chemotherapy. We will review each patient's clinical records, including their pathology report from biopsy and information about treatment. Malignancy will be determined as indicated by the pathology report.

4.5 Disease Progression

If signs of progression are captured during study assessments, the patient and her medical oncologist (with the patient's permission) will be notified. The non-FDA approved research images cannot be assumed to be of clinical quality and, therefore, cannot substitute for a clinical evaluation. Subjects experiencing symptoms for which clinical imaging may be appropriate will be advised to see their treating physician.

5.0 EXPECTED RISKS/UNANTICIPATED PROBLEMS/SAFETY MONITORING

5.1 Assessment of Safety

One aspect of this study outside the usual standard of care is the potential complication associated with the application of higher intensity ultrasound than is used in routine diagnostic examinations. If applied for extended time periods, these high-intensity beams could cause tissue damage due to excessive heating. The FDA considers thermal increases less than 6 ºC in soft tissue to be safe.[31] Therefore, the beam sequences and timing of data acquisition used for this study will be designed to ensure that the cumulative temperature increase does not exceed 2ºC, to further reduce patient risk and exposure. This is a research tool developed for academic purposes under the direction of Dr. Caterina Gallippi and is neither for commercial purposes nor under the direction of Siemens, the manufacturer of the ultrasound equipment.

The use of ARFI, VisR, and DDAI technology will build on experience from previous clinical studies conducted at UNC Chapel Hill (Gallippi, PI). IRB review designated these research ultrasound technologies as minimal risk and qualifying for abbreviated IDE (exempt from FDA review). No adverse events occurred. Our proposed research study is similar in nature to these trials, but if the IRB review determines that FDA review for an IDE is required, then an IDE application will

be submitted at that time. Other acoustic radiation force (ARF)-based imaging methods are approved for clinical practice in Europe and in the United States. Overall, the potential risks to participants would be categorized as minimal. Participants will be informed of these risks at the time of informed consent.

Given that the mechanical stresses during ARFI imaging are significantly less than those routinely experienced during both physiological arterial pulsation[32] and normal clinical evaluation by palpation, we believe this protocol poses no significant danger to the patient. Unexpected, unusual, or serious complications, or an unanticipated frequency of reactions during the course of the clinical investigation, will be reported immediately to the IRB.

The Principal Investigator, Co-Investigators, and Study Coordinators will have access to real time data on the rate of complications. If there is any increase in the rate of complications, we will review the entire protocol to seek solutions. Since the only addition to routine patient care associated with this study is ultrasound imaging that is believed to pose no significant risk to the patient, the chance of this happening is minimal. Any adverse events or unanticipated problems will be reported to the LCCC and the UNC IRB immediately.

5.2 Expected Risks (include Applicable Sections)

5.2.1 Patient Confidentiality

Identifiable information, which will be separated from research data, will be retained in a locked file cabinet, in a locked office, and password protected indefinitely. When the PI (Dr. Gallippi) determines that the identifiable information is no longer needed, it will be shredded (hard copy) and securely deleted (electronic). To achieve separation of identifiers from research data, all patient volunteers will be assigned unique study identification (ID) number at the time of enrollment. All acquired data will be labeled with the study ID number, and no personal information will be stored with the collected data. The master list linking study ID numbers to personal information will be secured by lock (hard-copy) and password (electronic copy) in Dr. Gallippi's laboratory in the UNC-CH Joint Department of Biomedical Engineering. Dr. Gallippi's computational work station is password-protected and locked in her research laboratory, so unauthorized personnel will not have access to personal identifiers. Any data transmission (by hard-copy or electronically) to authorized personnel will be stripped of any personal identifiers. The data will be tagged by the unique identifier assigned to the participant at the time of enrollment only. Electronically recorded data will be stored with study ID number only.

Except when required by law, study subjects will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of the University of North Carolina Hospitals, University of N.C. at Chapel Hill School of Medicine, and Departments of Radiology, Biomedical Engineering, and Oncology. No subject will be identified in any report or publication resulting from this study. All patient

volunteers will be assigned unique study identification (ID) number at the time of enrollment. All acquired data will be labeled with the study ID number, and no personal information will be stored with the collected data. The master list linking study ID numbers to personal information will be secured by lock (hard-copy) and password (electronic copy) in Dr. Gallippi's laboratory in the UNC-CH Joint Department of Biomedical Engineering. Dr. Gallippi's computational work station is password-protected and locked in her research laboratory, so unauthorized personnel will not have access to personal identifiers.

5.3 UNANTICIPATED CONCERNS

5.4 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)).

5.5 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.

5.6 Reporting

5.6.1 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.

5.7 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 criteria listed in section 5.6 must be reported to the UNC IRB using the IRB's web-based reporting system.

5.8 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.

6.0 STATISTICAL CONSIDERATIONS

6.1 Study Design/Study Endpoints

For Aims #1 and #2, the ability of ultrasound biomarkers to discriminate binary outcomes (malignant/bengin and responsive/unrespsonsive to treatment) will be analyzed using logistic regression. The area under the receiver operating characteristic curve (AUC) will be quantified, with separate models fit for each of the eight ultrasound biomarkers. Because most ultrasound biomarkers are continuous measures, the best-balanced cutpoints optimizing sensitivity and specificity will be calculated, using Youden's Index. Outcome discrimination by dual biomarkers will be analyzed in 28 separate models with combinations of any two ultrasound biomarkers. The combined benefit of ultrasound and mammography will be analyzed in 32 models, by adding a single mammography metric (lesion size, density, shape, or margins) to each of the eight ultrasound biomarker models. In Aim #2, the relationship between biomarker values and tumor size, biomarker values and change in tumor size, and change in biomarker values and change in tumor size will be analyzed by linear regression. These correlations will be analyzed in separate models for each of the eight ultrasound biomarkers, in models of dual ultrasound biomarkers, and in models combining mammography metrics with ultrasound biomarkers.

6.2 Sample Size and Accrual

A total of 80 participants will be recruited for this study. The study will be conducted in 40 women with diagnosed BIRADS-4 or -5 masses and in 40 women with malignant masses undergoing neoadjuvant chemotherapy.

We project 12 of the 40 women in our study will have malignant cancer, and 12 of 40 will have masses that do not respond to NAC. Given this, we expect 80% power to detect a minimum AUC of 0.70 (95% CI: 0.51 – 0.89). This is consistent with the diagnostic accuracy previously reported for malignancy detection by ultrasound biomarkers, which have ranged from AUC values of 0.67 to 0.99.[5,10]

6.3 Data Analysis Plans

6.3.1 Aim #1: Correlate ultrasound-derived stiffness, elasticity, viscosity, and anisotropy to malignancy.

In vivo ARFI, VisR, and DDAI imaging will be performed in 40 women with a breast mass with a BIRADS breast assessment categories of -4a, 4b, 4c, or -5 rating after diagnostic workup. First, the transducer will be placed lightly above the suspicious mass, just to the point of skin contact, to avoid pre-compression during imaging.[15] The suspicious mass will be positioned at 10 to 40 mm in axial depth, with standoff used if needed to achieve the desired axial position. Second,

the position of the transducer will be rotated through 90^o in steps of 30^o to identify the transducer position yielding maximum ARFI peak displacement in the mass. The transducer will be stabilized in this position, with light skin contact, by the sonographer or by using a stereotactic clamp. Third, ARFI and VisR data will be acquired in immediate succession. Fourth, the transducer will be rotated 90^o, re-stabilized by the sonographer or by using the stereotactic clamp, and ARFI and VisR data will be acquired in the new transducer position to enable DDAI analysis. Finally, the evaluated mass will undergo clinically indicated biopsy with histological evaluation to determine if it is benign or malignant.

The ultrasound data will be processed to calculate the following outcome metrics: peak displacement (PD), displacement at a given time (TD) for ARFI; τ, relative elasticity (RE), relative viscosity (RV), and tissue mass (TM) for VisR; ratio of ARFI PD and ratio of VisR RE values at 90^o versus 0^o transducer orientations for DDAI. These eight outcome metrics, alone and in combinations, will be statistically correlated with biopsy finding of malignant or benign.

6.3.1.1 Which of the 8 ultrasound metrics best detects malignancy?

The diagnostic accuracy of each ultrasound metric will be analyzed by constructing separate receiver operating characteristic (ROC) curves.

6.3.1.2 What is the combined diagnostic accuracy of any 2 ultrasound metrics?

The incremental value of using a second ultrasound metric will be analyzed by constructing separate ROC curves for combinations of any 2 ultrasound metrics.

6.3.1.3 What is the combined diagnostic accuracy of ultrasound metrics and mammography?

We will select ultrasound metrics with an AUC exceeding 0.75 in C.1.a-b, and their combined diagnostic accuracy with mammography measures (mass size, density, shape, and margins) will be analyzed in separate ROC curves.

6.3.1.4 Which cutpoints for ultrasound metrics detect malignancy with optimum sensitivity and specificity?

Best-balanced cutpoints from the highest performing ultrasound metrics in 6.3.1.1 will be derived from ROC curves, using Youden's Index. These cutpoints will be used to analyze the PPV.

6.3.2 Aim #2: Correlate ultrasound-derived stiffness, elasticity, viscosity, and anisotropy with response to treatment.

In 40 women with newly diagnosed invasive breast cancer who are scheduled to begin clinically indicated NAC, ARFI, VisR, and DDAI imaging will be performed (as described in Aim #1) at baseline, early within each chemotherapy type, and at the conclusion of each chemotherapy type over the course of treatment.

6.3.2.1 Which baseline ultrasound metric best predicts positive response to NAC?

Response to NAC will be dichotomized into a yes/no response according to the RECIST guidelines, and the predictive ability of each baseline ultrasound metric will be analyzed by constructing separate ROC curves.

6.3.2.2 Which baseline ultrasound metric correlates most strongly with absolute reduction in tumor size (diameter, area, volume)?

The relationship between ultrasound metric values at baseline and absolute change in tumor size from baseline to end of chemotherapy type will be visualized by scatterplots. If the relationship appears linear and monotonic, the correlation will be assessed by Pearson regression.

6.3.2.3 What is the combined ability of any 2 baseline ultrasound metrics for prediction of positive response to NAC?

With response to NAC dichotomized into a yes/no response, the combined predictive ability will be analyzed by constructing separate ROC curves for combinations of any 2 ultrasound metrics.

6.3.2.4 Do serial changes in ultrasound metrics correlate with reduction in tumor size following NAC?

The relationships between serial changes in ultrasound metrics and tumor size will be visualized by scatterplots. If monotonic and linear, the correlation between serial change in ultrasound metrics and change in tumor size (area) will be analyzed by Pearson regression. Assessments of serial change will be made at 3 intervals per chemotherapy type: baseline, early chemotherapy type treatment, and end of chemotherapy type treatment.

6.3.2.5 Serial change in which of the 8 ultrasound metrics best predicts positive response to NAC?

Response to NAC will be dichotomized into a yes/no response, and the predictive ability of serial change in each ultrasound metric from 0-6 weeks will be analyzed by constructing separate ROC curves.

6.3.2.6 Do combinations of changes over time in any ultrasound outcome metrics correlate with reduction in tumor size following NAC?

Assuming linear relationships exists between serial change in ultrasound metrics and serial change in tumor size following NAC, multiple linear regression models will be constructed for each of the 8 ultrasound metrics. The model outcome will be change in tumor size, and model predictors will be serial change in any 2 ultrasound metrics.

6.3.2.7 Which combinations of serial change for any 2 ultrasound metrics best predicts positive response to NAC?

Positive response to NAC will be dichotomized into a yes/no response, and the predictive ability of serial change in ultrasound metrics from baseline to end of

chemotherapy type will be analyzed by constructing separate ROC curves for combinations of any 2 ultrasound metrics.

6.3.2.8 What is the combined performance of ultrasound metrics and mammography for predicting a reduction in tumor size following NAC?

Reduction in tumor size will be dichotomized into a yes/no response. We will select ultrasound metrics with an AUC exceeding 0.75 in 6.3.2.1, 6.3.2.3, 6.3.2.5, and 6.3.2.7, and their combined diagnostic accuracy with mammography measures (mass size, density, shape, and margins) will be analyzed in separate ROC curves.

6.3.2.9 What are the sensitivity, specificity and PPV of ultrasound outcome metrics for identifying masses that positively respond to treatment?

Best-balanced cutpoints from the highest performing ultrasound metrics in 6.3.2.1 and 6.3.2.3 will be derived from ROC curves, using Youden's Index. These cutpoints will be used to analyze the PPV of positive response to NAC (dichotomized as a yes/no variable).

7.0 STUDY MANAGEMENT

7.1 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.

7.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be placed on file (as applicable).

- 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 sub-investigators who will be involved in the study.
- Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol College of American Pathologist (CAP) and Clinical Laboratory Improvement Amendments (CLIA) Laboratory certification numbers and institution lab normal values.

7.3 Registration Procedures

Patients will be registered into OnCore®, a web based clinical research platform by one of the Study Coordinators. The spreadsheet 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.

7.4 Data Management and Monitoring/Auditing

All data management will be performed by the senior graduate student research assistant working on this project, with supervision and guidance from the study PI, Dr. Caterina Gallippi. Automated methods are already in place to manage data acquisitions and storage. First, all raw data files acquired by the Siemens ultrasound system are labeled by the date and time of acquisition. Next, data files are stored into file folders labeled according to the assigned patient participant number 1-100 (which in no way reflects identifiable patient information). The data are processed using custom software developed in Matlab and/or C++, and the processed data files are stored in subfolders under each patient participant number. Analysis of the processed data often includes image rendering as well as assessment of contrast; contrast-to-noise; mean, median and standard deviation of relevant parameter values, etc. ROC analysis will also be performed. The results of data analysis will be stored with appropriate labeling indicating the corresponding patient number. Note that data storage will occur using secure UNC servers that meet the current standards for PHI security.

7.5 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.

7.5.1 Emergency Modifications

UNC and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

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.

7.5.2 Single Patient/Subject Exceptions

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

7.5.3 Other Protocol Deviations/Violations

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 meets any of the following criteria:

- 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 please follow the guidelines below:

Protocol Deviations: UNC personnel will record the deviation in OnCore®, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

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 UPIRSO.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSO): Any events that meet the criteria for "UPIRSO" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

7.6 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.

7.7 Record Retention

Study documentation includes all eCRFs, 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.

7.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with ethical principles originating from Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki, which are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) guidelines, and in accordance with applicable local and legal requirements. The Principal Investigator is responsible for personally overseeing the treatment of all study patients and ensuring that clinical data associated with biospecimen(s) collection should be used and disclosed only for research in compliance, as applicable, with HIPAA, with the U.S. Department of Health and Human Services and FDA human subjects protection regulations and with applicable State and local laws. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol in accordance with the guidance and regulations listed above both during and after study completion.

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

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