Clinical Study Protocol

Study Title:	Imagio Feasibility Multi-Reader, Multi-Case Study of Optoacoustic Images versus Imagio Ultrasound to Guide Decision to Biopsy
Study Type:	Single arm, controlled, blinded, multi-reader, multi-case study
Study Identifier:	Reader Study-01
Study Phase:	Feasibility
Study Objective:	To determine the design and sample size required for the pivotal study
Indication:	The Imagio™ breast imaging system is indicated for use by a trained and qualified healthcare provider for evaluation of breasts in women who are referred for a diagnostic breast work-up (including both palpable and non-palpable masses), following clinical presentation, mammography, diagnostic, screening, or staging methodology. In ultrasound mode, the device can be used to assess BI-RADS1-6. For BI-RADS 3-5 lesions, the ultrasound mode should be initially used to assess the lesion before moving to OA. In Opto-Acoustic (OA) mode, the Imagio™ provides information about the central nidus, boundary and peripheral zones to assist in the assessment of BI-RADS category and diagnosis of the benign or malignant lesion(s) of interest. Using OA and the corresponding features of the lesion(s) allows for improved classification of the mass of interest as compared to ultrasound alone in BIRADS 3-5 masses. This device is not intended to be used as a replacement for mammographic screening or for definitive pathologic diagnosis.
Sponsor:	Seno Medical Instruments, Inc.
Sponsor Contact:	Shaan Schaeffer, Vice President, Clinical Operations Seno Medical Instruments, Inc. 8023 Vantage Dr. Suite 1000 San Antonio, TX 78230 Phone: 210-615-6501 E-mail: sschaeffer@senomedical.com
IDE Approval Identifier	Not Applicable – Non-Significant Risk Study

PROTOCOL SIGNATURE PAGE

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TABLE OF CONTENTS

Page

List of Abbreviations and Definitions of Terms

ACR	American College of Radiology
AE	Adverse Event
ANOVA	Analysis of Variance
AUC	Area Under the Curve
BI-RADS	Breast Imaging-Reporting and Data System
CDU	Conventional Diagnostic Ultrasound
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture System
FDA	Food and Drug Administration
GEE	Generalized Estimating Equations
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITD	Intention-to-Diagnose
IUS	Imagio Internal Ultrasound
MRMC	Multi-reader, multi-case
N/A	Not Applicable
NLR	Negative Likelihood Ratio
NPV	Negative Predictive Value
NSR	Non-significant Risk
OA	Opto-Acoustic
PDU	Positive Diagnostic Ultrasound
PDUNB	Positive Diagnostic Ultrasound, No Biopsy
PIONEER	Abbreviated name of a previous study (Pivotal study of Imaging with Opto-acoustics to diagnose breast masses detected by mammography and/or clinical findings: A NEw Evaluation Tool for Radiologists)
PLR	Positive Likelihood Ratio
PMA	Premarket Approval
POM	Probability of Malignancy
PPV	Positive Predictive Value
QC	Quality Control
ROC	Receiver Operating Characteristic
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
USA	United States of America

STUDY TEAM LEADERS

Shaan Schaeffer - VP of Clinical Operations

Philip Lavin PhD, FASA, FRAPS - Chief Biostatistician

Tom Stavros MD, FACP - Chief Medical Officer

CLINICAL RESEARCH ORGANIZATIONS

American College of Radiology Center for Research and Innovation (ACR - CRI) will serve as the Imaging Core Lab to conduct the Imagio Feasibility Reader Study. ACR-CRI is located in Philadelphia, PA 19103. ACR-CRI complies with Good Clinical Practices (GCPs) and Title 21 Code of Federal Regulations (CFR) Part 11.

Boston Biostatistics Research Foundation (BBRF) will provide study design, mass sampling, database construction, and data analyses for the Imagio Feasibility Study. BBRF is located in Framingham, MA.

1. STUDY OBJECTIVES

1.1 Primary Objective

1. To select the effectiveness endpoints and determine the corresponding sample size required for the Pivotal study.

2. To evaluate the gain in *Imagio (Imagio Internal Ultrasound [IUS] + Opto-acoustic [OA])* specificity versus IUS, controlling for sensitivity

1.2 Secondary Objectives

To evaluate the following overall and for individual readers for IUS versus Imagio (OA+ IUS):

- the Imagio (IUS+OA) partial Receiver Operator Characteristic (ROC) Area under the Curve (AUC) for 95-99% vs IUS for 95-99% sensitivity

- the sensitivity and specificity for Imagio (IUS+OA) versus IUS according to multiple models for readers and masses used for assessing sources of variation

- the Negative Likelihood Ratio (NLR) for IUS versus Imagio (IUS+OA).

- the Imagio (IUS+OA) downgrades and upgrades relative to IUS, separately, for benign and malignant masses

- the net gain (new True Positives [TP] + new True Negatives [TN] – new False Positives [FP] – new False Negatives [FN]) estimate of Imagio (IUS+OA) versus IUS combining benign and malignant masses

- the usage and performance of the SenoGram model for the purposes of determining whether it will be adjusted prior to starting the pivotal study

1.3 Other Objectives

To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA):

- the differentiation between benign versus malignant masses with respect to Imagio (IUS+OA) feature scores

2. ENDPOINTS - endpoints to include a separate mammogram analysis

2.1 Primary Endpoint

To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA):

- the gain in Imagio (IUS+OA) specificity versus IUS for fixed sensitivity (e.g., 98%) with specificities interpolated from the respective ROC curve as applicable.

2.2 Secondary Endpoints

To evaluate the following overall and for individual readers for:

1. the partial ROC AUC corresponding to 95-99% sensitivity with specificities extrapolated from the respective Imagio and IUS ROC curves as applicable.
    - the observed and model-adjusted specificity and sensitivity
    - the Negative Likelihood Ratio (NLR) defined as ((1-sensitivity)/specificity)
    - the upgrade and downgrade metrics:
        - the percent correct of downgrades for benign masses from IUS BI-RADS 4a to Imagio (IUS+OA) BI-RADS 2-3, and from IUS BI-RADS 3 to Imagio (IUS+OA) BI-RADS 2
        - the percent incorrect of upgrades for benign masses from IUS BI-RADS 2-3 to Imagio (IUS+OA) BI-RADS 4-5 and from IUS BI-RADS 2 to Imagio (IUS+OA) BI-RADS 3-4-5
        - the percent of incorrect downgrades for malignant masses from IUS BI-RADS 4-5 to Imagio (IUS+OA) BI-RADS 2-3; and
        - the percent of correct upgrades for malignant masses from IUS BI-RADS 2-3 to Imagio (IUS+OA) BI-RADS 4-5.
2. the Cumulative effect of downgrades and upgrades for combined benign masses and malignant masses.
3. the SenoGram usage:
    - Utilization percent reported by the readers
    - Sensitivity and specificity discordance with Imagio (IUS+OA) Downgrades and upgrades versus Imagio (IUS+OA) and IUS, separately for benign and malignant masses
    - POM paired differences versus Imagio (IUS+OA) and IUS, separately for benign and malignant masses.
4. the SenoGram performance:
    - Sensitivity and specificity compared to sensitivity and specificity estimated from cross-validation and hold-out validation.
    - Partial AUC for individual users and overall corresponding to 95-99% sensitivity
    - Separate assessment of Senogram performance with and without mammographic data input.

2.3 Exploratory Endpoints

To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA):

- Imagio (IUS+OA) feature score distributions for benign versus malignant masses

3. BACKGROUND AND RATIONALE

The Imagio Feasibility Study is intended to evaluate if a subsequent pivotal study can be implemented to test prospective hypotheses for pre-specified effectiveness endpoints with an acceptable sample size. The Feasibility Study will be based on ITD masses from the PIONEER Pivotal study to simultaneously reflect the distributions with and without mammograms as well as site CDU BI-RADS scores within benign and malignant masses as strata.

4. STUDY DESIGN

This will be a single arm, controlled, blinded, multi-reader, multi-case (MRMC) study using a sequential design. The study will include 5-12 readers depending on qualifications and availability.

Imagio (IUS+OA) Training to be completed prior to any reads taking place. Read 1 is immediately followed by Read 2 within the same read session.

- Read 1 (Control): Mammogram (if available) + History + IUS (stills and videos provided), IUS Probability of Malignancy (POM) and BI-RADS scored and the data form locked.

- Read 2 (Test): Mammogram (if available) + History + IUS (stills and videos provided), and Imagio (IUS+OA) (stills and videos provided). An interim IUS POM and BI-RADS is recorded as well as a combined Imagio (IUS+OA) POM and BI-RADS Pre and Post SenoGram on separate data forms.

Read 1 reflects the information available to a radiologist when evaluating standard ultrasound images, taking under consideration the mass and patient history and assessing mammogram results as available. The History details to be provided are summarized under the Blinding section of the protocol. Read 1 (IUS reads) will serve as the control representing current clinical practice. Read 2 (IUS+OA) will be considered the test read and reflect a pre SenoGram IUS and Imagio (IUS+OA) POM and BI-RADS assessment in addition to a post SenoGram IUS and Imagio (IUS +OA) POM and BI-RADS assessment. Following the display of the SenoGram within the Post SenoGram data form, the IUS and OA feature scores are locked, and the reader is prompted to provide a Post SenoGram POM and BI-RADS. Once the Post SenoGram POM and BI-RADS are provided, the form is locked.

A total of 155 complete read sets from the original PIONEER Intention-to Diagnose (ITD) population will be randomly selected from within the PIONEER Study.

- Comprising the 155 mass read sets, the first cohort will consist of 75 benign and 45 malignant masses (reflecting the same 37.5% prevalence of cancer as for the overall pivotal ITD population), classified by conventional diagnostic ultrasound (CDU) as BI-RADS 3 to 5 to be selected at random in proportion to the original assignment distribution of BI-RADS classifications among subjects in the PIONEER Study. To facilitate the alignment of the PIONEER Pivotal Study data with the Feasibility Study data, the mass image set sampling plan will select a proportion with and without mammograms depending on availability of the mammograms, separately for benign and malignant masses. Cohort 1 will be used for the effectiveness endpoint analyses in support of decision making. The same sampling strategy will be used to ensure a comparable proportion of mass image sets as for the PIONEER Pivotal Study.

- The second cohort consists of 30 additional malignant masses for which 1 or more pivotal study readers scored the mass a false negative during a read from the PIONEER study; this will assess if the excess FN rate observed with OA in the PIONEER study can be reversed.
- The third cohort will consist of five DCIS, lymphoma, or Phyllodes masses; this will assess how these atypical cases will be scored. Since there are so few of these cases, mammogram matching will not be possible.

This evaluation will further achieve the following:

1. The incremental contributions of IUS and Imagio (IUS+OA) by assessing the BI-RADS and POM values at each step in the read order traditionally used in clinical practice.
2. The SenoGram performance versus IUS and versus Imagio (IUS+OA) once IUS + OA features are scored with subset evaluation of the effect of presence or absence of the mammographic data on Senogram performance.

The SenoGram is a classification model that predicts the probability that a lesion is malignant for a given set of reader-assigned feature scores and other relevant data. For this study, the SenoGram will consist of two components, a user interface and a back-end computation engine. The reader will enter feature data into the user interface. When all data has been entered, but prior to a final submission of the data, the user interface will graphically display the model's predicted likelihood of malignancy [not to be confused with POM].

The SenoGram figure will also display threshold lines that correspond to estimated sensitivities of 98%, 98.5%, 99%, and 99.5%, in relationship to the boundaries between BI-RADS categories. If the reader changes a feature score, the graphical display will automatically update with the revised prediction. The reader may optionally modify his or her BI-RADS and POM assignments based on the SenoGram predication; however, there is no requirement to do so. The use of the SenoGram result in decision making will be recorded for each reader for each image mass set evaluated. The SenoGram is trained on cases not selected for any Cohort of this study.

5. SELECTION OF READERS

5.1 Reader Qualification Criteria

- Completed residency and are board certified in radiology
- Active breast imager for at least 3 years.
- Readers to meet mammography interpretation requirements per mammography Quality Standards ACR (MQSA) for the year prior to study.
- Readers to meet breast imaging ultrasound interpretation requirements per ACR. for year prior to study.
- For the clinical study, a willingness to use BI-RADS 4 sub categories
- For the clinical study, the ability to participate and read all masses in both IUS and OA/US reader sessions.

5.2 Reader Training

Imagio (IUS+OA) Training Summary Reader 01 Study

1. Didactic training module *(4 hours)*
    a) Fundamentals of OA *(Questions and Answers)*
    b) OA feature scoring *(Questions and Answers)*
    c) OA-histologic correlation *(Questions and Answers)*
    d) OA artifacts and Scan Techniques *(Questions and Answers)*
    e) IUS feature scoring, including Importance of BI-RADS 4 subcategories, NLR and Bayes Theorem *(Questions and Answers)*
    f) Pivotal Study False Negative and False Positive analysis *(Questions and Answers)*
    g) The SenoGram and How to Use It
    h) Summary *(final questions and comments)*
2. Interactive reading case module *(estimate 5 cases per hour, 4-6 hours)*
    a) Mixture of benign and malignant enriched to 50% malignant
    b) Mixture of cases with good, average, and below average reader performance – will start with easy cases, move to average cases, and finish with most difficult cases
    c) Some malignant masses that caused false negative Pivotal study OA reads
    d) Readers will learn to use reading station, draw ROIs, score features, and use the OA and combined OA-IUS SenoGrams to predict OA POM and BI-RADS category and downgrade
    e) Each case will be read and scored by trainees, and then the Seno instructor will review how and why he/she would score the case, and finally review the histology and discuss concordance or discordance of OA feature scoring with the histology.
3. Test Cases - 30 (7.5 hours) 4 cases an hour
    a) Mixture of benign (18) and malignant (12) masses, same prevalence as overall Pivotal ITD population
    b) Mixture of easy, average, and difficult cases based upon Pivotal study reads
    c) Some masses that caused false negative pivotal study Imagio (IUS+OA) reads
4. Pass / Fail criteria

    Readers reading must pass a proficiency test before starting their study reads. If this is not achieved the first time the reader takes the test, the reader will be given targeted remediation training to their deviations and a second opportunity to take the test on a different set of test cases. The reader will proceed to read cases whether or not they pass the second test. If they fail the second test, remediation will take place before they start the study reads.

6. SELECTION OF READER SETS

6.1 Inclusion Criteria

Reader sets must meet all the following inclusion criteria to be included in this study:

- One analyzable mass per patient
- DCIS, lymphoma, and phyllodes masses to be looked at separately
- BI-RADS 3 and 4a, 4b, 4c and 5 masses as declared by clinical site investigator via PIONEER study conventional diagnostic ultrasound (CDU)
- Masses declared to be ITD
- Evaluable mammograms for a percentage of cases
- Patient age, indication and history available
- Evaluable OA and IUS video loops and stills

6.2 Exclusion Criteria

Reader sets must be excluded if any of the following criteria are met:

- Critical missing views for mammogram cases, IUS or OA stills and video that would preclude case from being evaluated by readers
- Mammogram, IUS or Imagio (IUS+OA) image quality inadequate due to error in labeling or positioning or acquisition technique
- Masses from multiple mass cases incorrectly labeled.
- Reader training cases

6.3 Reader Set Selection Procedure

- BBRF will select the mass sampling strategy taking training cases and cohort requirements into consideration
- BBRF will prepare the mass read order for Part 2 of the study.
- All image sets will be de-identified.

7.0 READER STUDY PROCEDURES

7.1 Reader Study Process Work Flow

Figure 1 below illustrates, at a high level, the central reader study process flow. Seno Medical will electronically transfer image sets to ACR-CRI. Upon receipt of the images at ACR CRI, imaging support staff will upload images to **Tr**ansfer of **I**mages and **D**ata [TRIAD], ACR's electronic image submission tool.

The images automatically undergo a de-identification process in TRIAD™, whereby all personal identifier DICOM tags in the image metadata are de-identified according to TRIAD™'s anonymization profile. If any personal identifiers are burned into exams received, ACR CRI staff will remove that by pixel cleaning. ACR-CRI imaging technologists will first perform quality control (QC-1) to document an inventory of all exams and their attributes.

ACR-CRI contracted multiple mass quality control reviewer will assess the image sets (QC-2) for inclusion by identifying and labeling Mass 1 on all modalities submitted for a given subject case where multiple masses are present. The acceptable image sets from the ACR QC (QC-1 and QC-2) process will be approved by BBRF and considered ready for central read.

BBRF will generate the study mass randomization scheme for the study.

The QC-1 and QC-2 checks will be documented in study specific procedure documents and outputs of such checks documented on study specific forms, all of which will be archived in an electronic Trial Master File (eTMF) and ACR CRI clinical databases.

ACR research staff serving as read monitors will be present during read sessions to ensure the read sequence is maintained, eCRFs are fully completed by the reader, cross-check the assigned read list with completed eCRFs and assist in any workstation-related questions. The read monitors will relay any issues they cannot resolve to the ACR Project Manager for remediation.

For the Feasibility Reader Study, a manual process of data entry will be performed. In order to maximize both workflow efficiency and data integrity, this process will be achieved by using the combination of the reader and a read monitor, working together as a team. Both the reader and the read monitor will be trained on the data entry process prior to production subject case assessments. The process of data entry is outlined below.

1. Read monitor calls out the Read ID to be reviewed based on the provided Reader Work List. The reader opens the Subject image case in the image viewing workstation. The read monitor opens the Subject eCRF case in the Rave Electronic Data Capture (EDC) Database. The reader calls out the Subject ID for the opened case as confirmation that it corresponds to the Subject ID opened in the eCRF database by the read monitor.
2. Reader performs the specified subject's image review using the designated Workstation and following assessment criteria as trained on for this study.
3. Read monitor prepares to perform data entry for the specified subject.
4. When the reader is ready to provide the data for data entry, the reader verbally notifies the read monitor.
5. The reader verbally provides the answers to each of the eCRF required field entries.
6. As the read monitor performs the manual data entry for each of the sequential fields directed by the flow of the eCRF, the read monitor verbally calls out their entry which is then verbally confirmed (or corrected, as necessary) by the reader.
7. This process will be repeated until all the appropriate fields for each of the required eCRFs are completed.

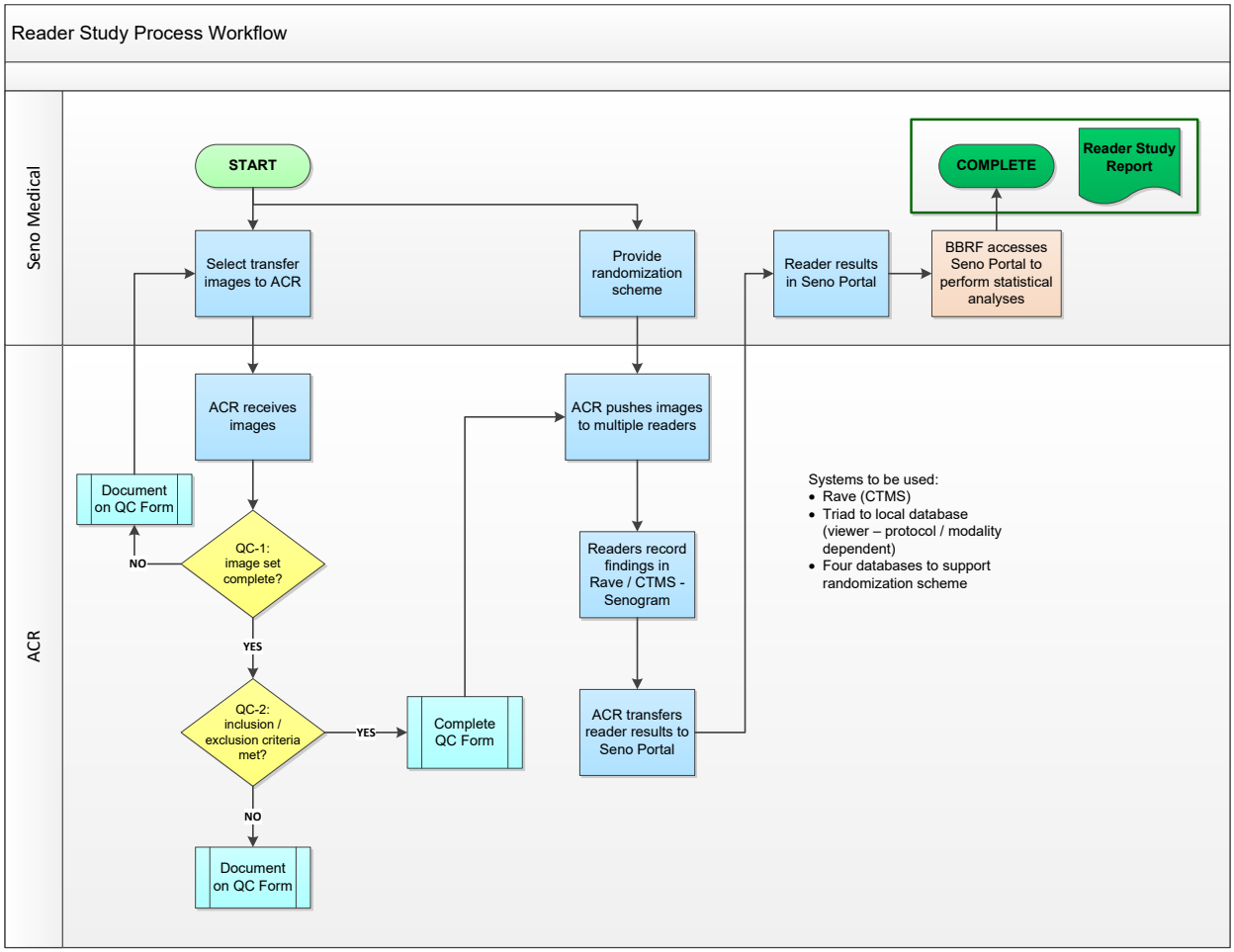
The reader electronically signs off on the data for a subject case at the completion of his/her review. It is understood that the commitment of a reader's electronic signature on the eCRF signifies that the assessment of the corresponding randomization ID is complete and accurate and is an attestation that no changes/edits need to occur.

The read monitor should be attuned to reader performance and suggest breaks as necessary to avoid reader fatigue.

As per the image review and data entry workflow described in this Protocol, once a subject scan review is completed by a reader, the reader provides their electronic signature and the eCRF is 'locked', preventing further changes.

Upon completion of all central read assessments by all readers, ACR-CRI will transfer the readers' results to Seno Medical's portal. BBRF will have access to the portal for purposes of conducting the statistical analyses.

Figure 1: Reader Study Work Flow



7.2 Schedule of Reader Sessions

All reads will include standard reader training on an image viewing workstation, eCRF completion, etc.

Imagio (IUS +OA) training and a proficiency test will take place prior to the start of all study reads.

7.3 Description of Reading Environment

After completion of standard training and Imagio (IUS+OA) training, each of the readers will use an image display and electronic data capture workstation to perform image analysis, working alongside an ACR Read Monitor, for all reading sessions. Each reader and read monitor will be in a designated, private reading room for their use during a reading session. Readers will work undistracted so that their attention is focused on an accurate interpretation. The workstation desk will have adjustable height for reader comfort, the room will be equipped with noise abatement features, diagnostic display monitors and moderate illumination. As part of reader training and as documented in the confidentiality agreement they sign during the ACR CRI contracting process, readers will be aware and monitored to maintain confidentiality on study details and subject images reviewed. Access to the reading rooms is controlled and read monitors ensure readers do not take any unauthorized material into the reading rooms.

7.4 Graphical Interface for BI-RADS Category and POM Rating;

For the purpose of this study, the Readers will use a graphical interface to indicate their assessment of the BI-RADS category and POM rating for IUS and Imagio (IUS+OA) modalities (see *Figure 2*).

The POM scale is linear for all BI-RADS categories; however, the scale for BI-RADS 3 is expanded ten-fold compared to other categories. This design allows for a finer gradation of the malignancy rating for BI-RADS 3 lesions, which in turn provides more data points for the ROC curve and enables a better estimation of the partial AUC. One advantage of a graphical metaphor is that it provides an aid to the Reader in converting the BI-RADS feature lexicon to a numerical POM scale.

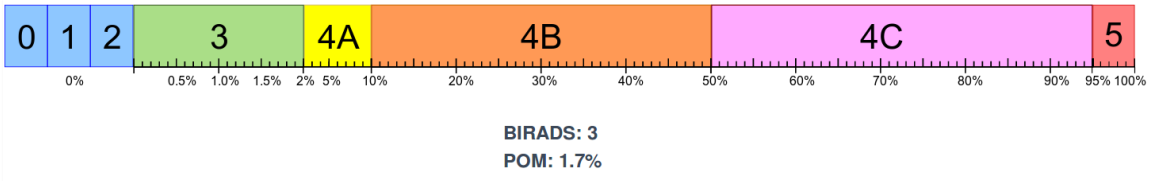


Figure 2. Graphical input of BI-RADS category and POM rating.

7.5 Randomization

BBRF will generate the randomized study read list.

7.6 Blinding

The readers will be blinded to the diagnosis, but the reader will have access to the following baseline variables:

- Age
- Indication (reason study was ordered)
- Mass location
- Medical History
- Mammograms (if available)

8. SAFETY ASSESSMENTS

Safety assessments are not required as this is a reader study of de-identified Imagio (IUS+OA) and IUS images. No patient diagnoses are affected by the reader sets or this MRMC study.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

BBRF will implement the Feasibility Study sampling plan to represent the PIONEER Pivotal Study masses to reflect mammography use and site CDU BI-RADS score.

BBRF will determine which masses are to be assigned to each cohort; Cohorts 1-3 will be the same across all readers.

The unit of measurement for reader-based outcomes is at the reader-mass level. The Feasibility Study will be used to assess if independent readers can conduct pre-specified POM evaluations separately for IUS and then for Imagio (IUS+OA) and the Senogram to evaluate the primary and secondary effectiveness endpoints. Pre-specified POM evaluations will be performed separately for IUS, Imagio (IUS+OA), and the reader use of the SenoGram to evaluate the Imagio (IUS+OA) and SenoGram specificities and sensitivities.

The primary endpoint is to show a specificity advantage for fixed sensitivity; this diffuses the tradeoff between FNs and FPs if the FN rate is the same. These sensitivity and specificities will be calculated overall and per reader using generalized estimating equations (GEE) and the analysis of variance (ANOVA) modeling as well as for observed without adjustment.

For each endpoint, Imagio will be compared versus IUS; the same analyses will be performed for the SenoGram versus IUS and Imagio.

The specificity advantages will be computed for fixed sensitivity (e.g. 98% target) with interpolation or extrapolation used by calculating the specificities and sensitivities from the ROC curve as needed. This same principle will be used to construct the partial ROC curves and to compute the area under the ROC curve to correspond to 95-99% sensitivity.

The results from the Feasibility Study will be used to judge if there is sufficient rationale to proceed to a pivotal study involving more masses and more readers to support the indications for use. If there is sufficient strength of the data, then the Imagio Feasibility Study results will be used to determine the pivotal study sample size. The effect sizes will be assessed to judge if the pivotal study can be conducted with at least 80% power for two-sided hypothesis testing with 5% Type I error; this depends on the effect sizes achieved for the primary and secondary effectiveness endpoints as well as inter-reader variabilities.

Feasibility Study data will not be used or combined with any subsequent pivotal study data, but the same masses used in the Feasibility Study may be used in the subsequent pivotal study.

The Feasibility Study will not test any formal statistical hypotheses but will be used to formulate hypotheses to be formally tested in the pivotal study.

A move forward decision will be made based on the collective performance of the primary and secondary effectiveness endpoints using Cohort 1 (75 benign masses and 45 malignant masses). Cohort 2 will give separate confidence that training has helped to reduce FNs. Cohorts 1, 2 and 3 will be analyzed separately.

9.2 Reader Set Sample Size

This Imagio Feasibility Study is not powered but should be more than adequate to estimate effect sizes to power the subsequent pivotal study.

Every effort will be made to identify missing reads and to provide readers with the opportunity to read all 155 image sets which includes the two additional cohorts:

- Cohort 1: 120 masses (75 benign, 45 malignant) to represent the PIONEER study
- Cohort 2: 30 additional malignant masses with FNs
- Cohort 3: 5 DCIS, lymphoma and phyllodes masses.

All reads will be included in all analyses, consistent with an “intention to diagnose” approach.

9.4 Population Definition

The original PIONEER Intention-to-diagnose classification will be used. Images will be selected for use in accordance with the study protocol inclusion and exclusion criteria.

9.5 Interim Analyses

An interim analysis of the data is planned to follow a completion of a minimum of 72 completed Cohort 1 mass reads by each reader in Part 2 to assess intermediate performance results.

9.6 Protocol Deviations

The study data will be evaluated for major and minor protocol deviations. This will be limited to missing and incomplete reads from the Feasibility Study.

At study completion, the number and type of protocol deviations will be analyzed by the study biostatistician to review the events by reader to determine whether there are statistical concerns. The biostatistician will report findings to the study sponsor.

The findings will be used to improve the pivotal study design in the event that the pivotal study is to proceed.

9.7 Outcomes

Outcomes will be analyzed according to the PIONEER Truth Panel findings and the pathology diagnosis (if available) as truth; Truth Panel Benign (TPB) will be a benign diagnosis. The final analyses will be performed following database lock.

9.8 Data Analyses

All analyses will be performed in a GCP-controlled environment using SAS v9.3 or higher and StatXact v10 or later unless otherwise specified.

All significance testing will be two-sided; results will be presented using two-sided p-values and two-sided 95% confidence intervals. All p-values are considered to be descriptive.

Methods of analysis for the MRMC design will be analyzed treating readers as random effects and treatment and masses as fixed effects and also by MRMC analysis methods by Obuchowski and Rockette where both reader and mass are random effects (see references). Two-sided 95% confidence intervals will be computed for the Imagio (IUS+OA) as well as Imagio (IUS+OA) - versus IUS differences in ROC AUC and the differences in downgrade and upgrade percentages separately for benign and malignant masses. The same analyses will be performed for the SenoGram versus IUS and versus Imagio (IUS+OA). These outcomes will be used to guide decisions regarding pivotal study design.

For each endpoint, Cohort 1 subgroup analyses will be performed in the same manner for those with a prior mammogram as well as for those without a prior mammogram.

1. Sensitivity, Specificity, Partial ROC AUC, and Specificity for Fixed Sensitivity

Individual reader specificity and sensitivity will be calculated from simple counts for POMs to construct the respective ROC curves for IUS, the Imagio (IUS+OA) and the SenoGram. GEE and ANOVA will be used to describe overall specificity and sensitivity as well as to compute the two-sided 95% confidence intervals for the OA-IUS differences for sensitivity and for specificity.

The specificity advantage for fixed sensitivity will be the primary endpoint. It will be computed from the respective ROC curves which are generated according to the POMs corresponding to the 95-99% sensitivity range. To compute the specificity advantage for fixed sensitivity, interpolation may be required; a fixed sensitivity will be targeted, e.g., 98%. To compute the specificity advantage and the partial area under the ROC, the specificities and sensitivities may need to be further computed from the ROC curves as needed. The McClish method will be used to analyze and compare the partial ROC curves. The variances will be computed to construct the two-sided 95% CIs for Imagio (IUS+OA), IUS, and for the Imagio (IUS+OA) -IUS difference. The same analyses will be performed for the SenoGram versus IUS and versus Imagio (IUS+OA).

In addition, the interpolation and the corresponding variance estimate will also permit the calculation of the specificity difference for fixed sensitivity (e.g. 98%) as well as the two-sided 95% confidence interval for the difference.

2. Upgrades and Downgrades

Separately for benign + TPB masses and malignant masses, downgrades and upgrades will be jointly assessed for each diagnostic category using a McNemar paired comparison test for Imagio (IUS+OA) versus IUS. In addition, two-sided 95% confidence intervals will be computed for the OA-IUS differences for both specificity and sensitivity; superiority will be sought for specificity while non-inferiority will be sought for sensitivity. Refer to Table 1 below.

Table 1: Upgrades and Downgrades

	Imagio (IUS+OA) Clinical Significance Criteria	Imagio (IUS+OA) Statistical Significance Criteria
Benign + TPB Downgrades	BR4a→BR2-3; BR3→BR2	Establish an advantage
Benign + TPB Upgrades	BR2-3→BR4-5; BR2→BR3-4-5	Rule out a disadvantage
Malignant Upgrades	BR2→BR4-5; BR3→BR4-5	Establish an advantage
Malignant Downgrades	BR4→BR2-3; BR5→BR2-3	Rule out a disadvantage

Supporting descriptive statistics will be computed as follows for the correct downgrades and incorrect upgrades for benign masses. Refer to Table 2 below.

Table 2: Correct Downgrades and Incorrect Upgrades

Imagio (IUS+OA) Outcome (IUS→Imagio)	Benign + TPB Masses
BR4a→BR3	% (IB3/IB4a)
BR4a→BR2	% (IB2/IB4a)
BR3→BR2	% (IB2/IB3)
Correct Downgrade	% (IBN/IBD)
Imagio (IUS+OA) Outcome (IUS→Imagio)	**Benign + TPB Masses**
BR2→BR3+	% (IB3-5/IB2)
BR3→BR4a+	% (IB4-5/IB3)
Incorrect Upgrade	% (IBN/IBD)

Supporting descriptive statistics will be computed as follows for the correct upgrades and incorrect downgrades for malignant masses. Refer to Table 3 below.

Table 3: Correct Upgrades and Incorrect Downgrades

Imagio (IUS+OA) Outcome (IUS→Imagio)	Malignant Masses
BR2→BR4-5	% (IC4-5/IC2)
BR3→BR4-5	% (IC4-5/IC3)
Correct Upgrades	% (ICN/ICD)
Imagio (IUS+OA) Outcome (IUS→Imagio)	**Malignant Masses**
BR4->BR2-3	% (IC2-3/IC4)
BR5->BR2-3	% (IC2-3/IC5)
Incorrect Downgrades	% (ICN/ICD)

To assess the cumulative effect of downgrades and upgrades for combined benign masses and malignant masses, a net gain will be computed for downgrades and upgrades. The net gain will be computed in SAS according to contrast statements for the ANOVA models to simultaneously analyze reader and mass. The weight (W) of the malignant outcomes (TP and FN) will be computed to determine the value (A-B+W(C-D)) that achieves statistical significance. The same analyses will be performed for the SenoGram versus IUS and versus Imagio. Refer to Table 4 below.

Table 4: Net Gain

	Imagio (IUS+OA) Relative to IUS
Benign Masses Picked Up	A
Benign Masses Lost	B
Malignant Masses Picked Up	C
Malignant Masses Lost	D
Numeric Gain	A-B+C-D

3. Move Forward Criteria

The move forward criteria will be dependent on data from the above endpoints. As illustration, the following criteria would justify conducting the Pivotal Study:

- An Imagio (IUS+OA) specificity advantage versus IUS for fixed sensitivity;
- An Imagio (IUS+OA) specificity advantage with a minimal OA sensitivity disadvantage;
- An Imagio (IUS+OA) advantage for benign mass downgrades with a minimal Imagio (IUS+OA) disadvantage for malignant mass upgrades.

The same analyses will be performed for the SenoGram versus IUS and versus Imagio. Depending which criteria are selected for inclusion as pivotal study primary endpoints, the pivotal study will be designed to have 80% power with two-sided 5% Type I error; there are 1,757 masses available from the PIONEER Pivotal Study.

4. NLR

The NLR ((1-sensitivity)/specificity) will be computed using the standard formula with two-sided 95% CIs computed using exact 2x2 contingency table methods. A two-sided 95% CI for the NLR <1 and favoring OA will indicate improvement in NLR; the relative NLR will also be computed relative to IUS. The same analysis will be performed for SenoGram versus IUS and versus Imagio (IUS+OA).

5. Graphical Displays

The BI-RADS findings for Imagio and SenoGram will be displayed separately for benign masses using three separate scales (0-2%, 2-50%, 50-100%) to better represent reader opinions and Senogram outcomes. The 0-2% scale will be on an expanded scale to allow the readers to more precisely identify the respective POM for Imagio and likelihood of malignancy for the SenoGram.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Management

Independent read data for this study is populated in Medidata Rave EDC System via direct data entry during read sessions from readers and read monitor staff. Electronic data collection forms in Rave will be built during study start-up and designed to meet study aims and statistical analysis needs. Electronic case report forms are created based on the study protocol, ACR-CRI scope of work statement, contract, data flows, and data collection needs. The ACR-CRI Data Manager will ensure the eCRFs are tested appropriately, maintain proper version control and all internal and external/Sponsor stakeholders approve the content prior to finalization.

10.2 Quality Assurance

10.2.1 Training

Reader training will be provided as described in section 5.2 above.

10.2.2 Quality Control

Monitoring of the readers will be done by ACR in accordance with the procedures outlined under the Reader Study Process Work Flow Section of the protocol and the Independent Review Charter.

10.3.4 Monitoring

Monitoring methods for assuring data quality for each reader, accurate qualification of each reader and reader responsibilities during the read process will be documented in the Independent Review Charter.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Informed Consent Forms

Not applicable for this study.

11.2 Participant Confidentiality

Reader sets will be de-identified to maintain PIONEER study participant's confidentiality according to the Health Insurance Portability and Accountability Act (HIPAA), any special data security requirements as stipulated by participating readers, and record retention per the sponsor's requirements.

Any data, forms, reports, video recordings, and other records that the participating reader receives from the sponsor will be identified only by a participant identification number (Participant ID, PID) from the PIONEER study to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by the FDA.

11.3 Study Discontinuation

The study may be discontinued at any time by the sponsor for futility or by FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript will be made available for review by the sponsor prior to submission.

Study results may be published in support of reimbursement.

Seno will review and approve all manuscripts.

13. REFERENCES

1. *Dorfman, D.D., Berbaum, K.S., & Metz, C.E. (1992). Receiver operating characteristic rating analysis: Generalization to the population of readers and patients with the jackknife method. Investigative Radiology, 27, 723-731.*

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3. *Hillis, S.L., Obuchowski, N.A., Schartz, K.M., & Berbaum, K.S. (2005). A comparison of the Dorfman-Berbaum-Metz and Obuchowski-Rockette methods for receiver operating characteristic (ROC) data. Statistics in Medicine, 24, 1579-1607 DOI:10.1002/sim.2024.*

4. *Hillis, S.L. (2007). A comparison of denominator degrees of freedom for multiple observer ROC analysis. Statistics in Medicine, 26:596-619 DOI:10.1002/sim.2532.*

5. *Hillis, S.L., Berbaum, K.S., & Metz, C.E. (2008). Recent developments in the Dorfman-Berbaum-Metz procedure for multi-reader ROC study analysis. Academic Radiology, 15, 647-661. DOI:10.1016/j.acra.2007.12.015.*

14. VERSION HISTORY

Version	Date	Description of Changes
1	27 June 2018	Original protocol
2	24 Sept 2018	Section 1.1 - Primary Objectives - revised to include 1. To select the effectiveness endpoints and determine the corresponding sample size required for the Pivotal study. 2. To evaluate the gain in *Imagio (Imagio Internal Ultrasound [IUS] + Opto-acoustic [OA])* specificity versus IUS controlling for sensitivity CDU benchmark classification objectives were removed. Section 1.2 - Secondary Objectives- revised to include: • the Imagio (IUS+OA) partial Receiver Operator Characteristic (ROC) Area under the Curve (AUC) corresponding to the IUS sensitivity over 95-99% sensitivity • the sensitivity and specificity for Imagio (IUS+OA) versus IUS according to multiple models for readers and masses used for assessing sources of variation • the Negative Likelihood Ratio (NLR) for IUS versus Imagio (IUS+OA). • Imagio (IUS+OA) downgrades and upgrades relative to IUS for benign and malignant masses • the net gain (new True Positives [TP] + new True Negatives [TN] – new False Positives [FP] – new False Negatives [FN]) estimate of Imagio (IUS+OA) versus IUS combining benign and malignant masses. • the usage and performance of the SenoGram model for the purposes of determining whether it will be adjusted prior to starting the pivotal study Section 1.3 - Other Objectives- revised to include: To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA): • the differentiation between benign versus malignant masses with respect to Imagio (IUS+OA) feature scores Section 1.3 - Other Objectives- revised to include:

Version	Date	Description of Changes
2, cont.	24 Sept 2018	To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA): • the differentiation between benign versus malignant masses with respect to Imagio (IUS+OA) feature scores Section 2.1 - Primary Endpoints - revised endpoints to correspond to objectives Section 2.2 - Secondary Endpoints - revised endpoints to correspond to objectives CDU benchmark classification endpoints were removed. Section 2.3 – Exploratory Endpoints – revised endpoints to correspond to objectives and added mammography endpoint Section 4 – Study Design- modified to sequential design. 5 -10 readers to read both Part 1 and 2 of the study with a minimum 30-day wash-out period in between SenoGram- language added: “When all data has been entered, but prior to a final submission of the data, the user interface will graphically display the model’s predicted likelihood of malignancy [Not to be confused with POM]. The SenoGram figure will also display threshold lines that correspond to estimated sensitivities of 98%, 98.5%, 99%, and 99.5%, in relationship to the boundaries between BI-RADS categories. “Critically, the SenoGram is trained on cases not selected for Cohort 1 of this study”. Section 5.1 Reader Qualification Criteria Removed criteria “ that is at least 70% breast imaging” Added criteria: Ability to participate and read all masses in both IUS and OA/US reader sessions Section 5.2 – Reader Training – revised and re-ordered training sections adding Question and Answer sections Part 4 revised to: Readers reading Part 2 must pass a proficiency test before starting their Imagio (IUS+OA) reads. If this is not achieved the first time the reader takes the test, the reader will be given targeted remediation training to their deviations and a second opportunity to take the test on a different set of test cases. If the reader does not pass the second time, they will be dropped from the study participation and not continue with Part 2 reads. In the case of dropped readers that do not pass the proficiency test or discontinue the study for any other reason (e.g., illness, personal emergency), then the IUS data for those readers will be analyzed separately.

Version	Date	Description of Changes
2, cont.	24 Sept 2018	Section 6.2 Exclusion criteria Exclusion criteria modified to: • Critical missing views for mammogram cases, IUS or OA stills and video that would preclude case from being evaluated by readers Section 6.3 Reader Set Selection Criteria The following was added: • BBRF will prepare the mass read order for Part 2 of the study. Section 7.1 Reader Study Process Work Flow The following language was modified to read: "The images automatically undergo a de-identification process in TRIAD™, whereby all personal identifier DICOM tags in the image metadata are de-identified according to TRIAD™'s anonymization profile. If any personal identifiers are burned into exams received, ACR CRI staff will remove that by pixel cleaning. ACR-CRI imaging technologists will first perform quality control (QC-1) to document an inventory of all exams and their attributes. ACR-CRI contracted multiple mass quality control reviewer will assess the image sets (QC-2) for inclusion by identifying and labeling Mass 1 on all modalities submitted for a given subject case where multiple masses are present. The acceptable image sets from the ACR QC (QC-1 and QC-2) process will be approved by BBRF and considered ready for central read. BBRF will generate the study's mass randomization scheme for Part 2 of the study. Masses will be randomized separately for Part 1 and Part 2 to keep readers blinded to the fact that they are reading repeat cases. The QC-1 and QC-2 checks will be documented in study specific procedure documents and outputs of such checks documented on study specific forms, all of which will be archived in an electronic Trial Master File (eTMF) and ACR CRI clinical databases." "BBRF will generate the study's mass randomization scheme for Part 2 of the study". Section 7.2 Schedule of Reader Sessions Section 7.2 was modified to accommodate 2 parts to study: Part 1 will include standard reader training on an image viewing workstation, eCRF completion, etc. There will be a minimum 30-day wash-out period in between the reader's last part 1 read session and the reader's start of part 2 read sessions.

Version	Date	Description of Changes
2, cont.	24 Sept 2018	Part 2 will include Imagio (IUS+OA) training and a proficiency test. The same 5-10 study readers from Part 1, will read Part 2 If a reader fails the proficiency test and re-test, they will be dropped from the study at that time. Section 7.3 Description of Reading Environment The first sentence was modified to: After completion of standard training and Imagio (IUS+OA) training (as applicable), each of the readers will use an image display and electronic data capture workstation to perform image analysis, working alongside an ACR Read Monitor, for all reading sessions. Section 7.4 Randomization The following sentence was modified to: BBRF will generate randomized mass list for Part 2 of the study. Section 7.5 Blinding • Mammograms (if available) was added Section 9 – Statistical Considerations was rewritten to accommodate revised study objectives and endpoints Section 9.1 General Design Issues Modified to accommodate revised objectives and endpoints Section 9.5 Interim Analyses The following sentence was modified to: An interim analysis of the data is planned to follow a completion of a minimum of 72 completed Cohort 1 mass reads by each reader to assess intermediate performance results. Section 9.6 Protocol Deviations The following sentence was modified to: The study data will be evaluated for major and minor protocol deviations. This will be limited to missing and incomplete reads from the Feasibility Study. Artifact reference was removed. Section 9.8 Data Analyses This section was modified to accommodate the revised study objectives and endpoints Multiple formatting and administrative changes were made throughout the document

Version	Date	Description of Changes
3	01Nov2018	Protocol Cover Page - Indication for Use, modified to: The Imagio™ breast imaging system is indicated for use by a trained and qualified healthcare provider for evaluation of breasts in women who are referred for a diagnostic breast work-up (including both palpable and non-palpable masses), following clinical presentation, mammography, diagnostic, screening, or staging methodology. In ultrasound mode, the device can be used to assess BI-RADS1-6. For BI-RADS 3-5 lesions, the ultrasound mode should be initially used to assess the lesion before moving to OA. In Opto-Acoustic (OA) mode, the Imagio™ provides information about the central nidus, boundary and peripheral zones to assist in the assessment of BI-RADS category and diagnosis of the benign or malignant lesion(s) of interest. Using OA and the corresponding features of the lesion(s) allows for improved classification of the mass of interest as compared to ultrasound alone in BIRADS 3-5 masses. Section 1.2 Secondary Objectives, modified first bullet to: • the Imagio (IUS+OA) partial Receiver Operator Characteristic (ROC) Area under the Curve (AUC) for 95-99% vs IUS for 95-99% sensitivity • Deleted last bullet: the graphical display of BI-RADS 2-3 findings on expanded scales for the purposes of demonstrating Imagio and SenoGram benefit for downgrading benign masses Revised : 2. ENDPOINTS - endpoints to include a separate mammogram analysis Section 2.1 Primary Endpoint, modified to: To evaluate the following overall and for individual readers for IUS versus Imagio (IUS+OA) Section 2.2 Secondary Endpoints Item 2 modified to: the Cumulative effect of downgrades and upgrades for combined benign masses and malignant masses Item 3 – Senogram Usage: modified to Sensitivity and specificity discordance with Imagio (IUS+OA) Section 4 Study Design: this section was revised to reflect the changes to the study design. Section 5.1 Reader Qualification: this section was revised to reflect less stringent qualification criteria. Section 5.2 Reader Training: Item 1 – Didactic Training: deleted review of previous studies (PIONEER and MASETRO)

Version	Date	Description of Changes
3, cont.	01Nov2018	Item 4 – Pass / Fail Criteria: this section was modified to reflect change in pass/fall criteria Section 7.1 Reader Study Process Work Flow, modified third paragraph to: BBRF will generate the study mass randomization scheme for the study. Section 7.2 Schedule of Reader Sessions: this section was revised to reflect the changes in the study design. Section 7.4 Graphical Interface for BI-RADS Category and POM Rating: this section was modified to clarify its usage in the study Section 7.5 Randomization, modified to BBRF will generate the randomized study read list. Section 9.8 Data Analyses, added: item 5 Graphical Displays Multiple formatting changes and typo corrections throughout the document