Seno Medical Instruments, Inc.

Study Title:	Clinical Evaluation of Opto-Acoustic Image Quality with the Gen 2 Imagio System
Protocol Number:	GEN 2 Imaging-01
Study Device:	Gen 2 Imagio® Breast Imaging System
Sponsor:	Seno Medical Instruments, Inc.

Sponsor Contact/Representative:
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Study Duration: The study duration for each subject will be a single visit unless imaging is to be done again.

Date: 10 Nov 2021
Protocol Version: 4.0

PROTOCOL SIGNATURE PAGE

Clinical Investigation: Clinical Evaluation of Opto-Acoustic Image Quality with the Gen 2 Imagio System

Reference: GEN 2 Imaging-01

Signature Statement
I have reviewed this clinical investigation protocol describing the design and specific provisions of the clinical investigation. I agree with the content of this document.

Shaan Schaeffer
Vice President of Clinical Operations

11/10/2021
Date

A. Thomas Stavros, M.D.
Chief Medical Officer

Date

Ann Waterhouse, RAC
Vice President of Regulatory Affairs and Quality Assurance

Date

Pam Otto, M.D.
Chairman of the Department of Radiology

Date

Elizabeth Kane, PhD
Senior Biostatistician, Avania

Date

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A. Thomas Stavros, M.D. Chief Medical Officer	11-Nov-2021 Date
Ann Waterhouse, RAC Vice President of Regulatory Affairs and Quality Assurance	Date
Pam Otto, M.D. Chairman of the Department of Radiology	Date
Elizabeth Kane, PhD Senior Biostatistician, Avania	Date

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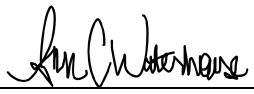	12Nov2021
Ann Waterhouse, RAC Vice President of Regulatory Affairs and Quality Assurance	Date
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Pam Otto, M.D.
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15-Nov-2021
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Ann Waterhouse, RAC Vice President of Regulatory Affairs and Quality Assurance	Date
Pam Otto, M.D. Chairman of the Department of Radiology	Date
Elizabeth Kane Elizabeth Kane, PhD Senior Biostatistician, Avania	10Nov2021 Date

INVESTIGATOR SIGNATURE PAGE

Clinical Investigation: Clinical Evaluation of Opto-Acoustic Image Quality with the Gen 2 Imagio System

Reference: GEN 2 Imaging-01

Investigator's Statement
I agree to conduct this clinical investigation in accordance with the design and specific provisions of this clinical investigation plan; modifications to the clinical investigation are only acceptable with a mutually agreed upon clinical investigation plan amendment as approved by the Sponsor and involved IRB.
I agree to await IRB approval of the clinical investigation plan and informed consent form before initiating the clinical investigation, to obtain consent from subjects prior to their enrollment, to collect and record data as required by the clinical investigation plan and associated case report forms, and to maintain documents related to the clinical investigation for the period of time required.

Confidential
This document contains confidential information belonging to Seno Medical Instruments, Inc. Except as may be otherwise agreed to in writing, by accepting or reviewing these materials, I agree to hold such information in confidence and not to disclose it to others (except where required by applicable law), nor use it for unauthorized purposes.

Investigator name (print)

Signature Date

SYNOPSIS

Clinical investigation plan	
Title	Clinical Evaluation of Opto-Acoustic Image Quality with the Gen 2 Imagio System
Short title	GEN 2 Imaging-01
Investigational device	
Name	GEN 2 Imagio® Breast Imaging System
Indication for Use	The Imagio Breast Imaging System is indicated for use by a trained and qualified healthcare provider for evaluation of palpable and non-palpable breast abnormalities in adult subjects who are referred for a diagnostic imaging breast work-up, following clinical presentation or either screening or diagnostic mammography or other imaging examinations. The ultrasound mode should be initially used in a targeted fashion, to assess any focal area(s) of clinical or imaging concerns. In ultrasound (US) mode, the device can be used to assign a BI-RADS (Breast Imaging-Reporting and Data System) category to either breast tissue or a mass that is causing clinical or imaging concerns. Masses that are classified as BI-RADS categories 3 through 5 can then be assessed using the Opto-Acoustic (OA) mode. In the OA mode, the Imagio Breast Imaging System provides information about the central nidus, boundary and peripheral zones, based on vascularity and blood oxygen saturation of the imaged tissues, to assist in the diagnosis of the benign or malignant mass(es) of interest. For ultrasound BI-RADS 3-5 masses, using the OA features of the mass allows for improved classification of the mass of interest as compared to ultrasound alone. The OA mode is not indicated for ultrasound BI-RADS 1 and 2 findings. The Imagio Breast Imaging System includes an artificial intelligence (AI) based software function to assist the users in assessing the BI-RADS Classifications. This device is not intended to be used as a replacement for mammographic screening or for definitive pathologic diagnosis.
Sponsor	
Name	Seno Medical Instruments, Inc. 8023 Vantage Drive, Suite 1000 San Antonio, TX 78230 United States of America
Contact details	Shaan Schaeffer Vice President of Clinical Operations +1 210 615 6501

Investigation centers	
Number of centers	One (1)
Location of centers	USA
Clinical investigation design	
Objectives	1. Obtain ultrasound only probe images and duplex probe OA/US (both gray scale and OA) probe images. This includes doppler and elastography imaging with the gray scale only ultrasound probe and ultrasound mode of the duplex OA/US probe. 2. Provide breast and lymph node pathology results (except BI-RADS 1, 2 and 3 (as applicable))
Endpoints	- Imagio (IUS+OA) vs Pathology Result assessment - Adverse Event Assessment
Intervention	Each subject will have the breast and, as applicable, the axillary lymph nodes imaged with the Gen 2 Imagio system.
Duration of study visit	One visit of up to 60 minutes unless Imagio imaging needs to be done again
Total duration	6 months
Clinical investigation population	
Sample size	A maximum of 38 healthy subjects ≥18 years old will be enrolled in the study. The 38 subjects will include the following: - 4 BI-RADS 1 - 4 BI-RADS 2 - 8 subjects with BI-RADS 3 - 8 subjects with BI-RADS 4a - 4 subjects with BI-RADS 4b - 4 subjects with BI-RADS 4c - 6 subjects with BI-RADS 5
Inclusion criteria	Subjects must meet the following criteria: - Signed and dated informed consent, prior to participating - At least 18 years of age - Have been referred for a breast US because of a breast lesion/mass finding via a palpable lump or per standard of care imaging - Willing and able to comply with protocol required scans
Exclusion criteria	Subjects who meet any of the following criteria will be excluded: - Pregnant or lactating - Has a condition or breast impediment at a location where the duplex probe will be in contact with the subject, which could interfere with the intended field of view - Photo-toxicity associated with currently taking, or having taken, photosensitizing agents within the previous 72 hours - Currently undergoing phototherapy - History of photosensitive disease or undergoing treatment for a photosensitive disease and is experiencing photosensitivity

	- Previous adverse reaction to medical laser procedures - Prior benign excisional breast biopsy on breast of interest within the past 18 months - Current mastitis - -Prisoners
Statistical analysis	
Analysis sets	Full Analysis Set (FAS) Population The FAS population will be defined as all consented subjects who completed screening/enrollment and had a successful Imagio imaging procedure. The FAS population will be used as the secondary analysis population for the study endpoints. Per-protocol (PP) Population The PP population will be defined as all FAS subjects described above who meet the study eligibility criteria and have no other major protocol violations. The PP population will be used for primary analysis of the study endpoints. Safety Population The safety population will be defined as all subjects in whom the Imagio imaging procedure was attempted. This analysis population is the primary population for all safety analyses.
Statistical design	Summaries of each study endpoint and other clinical/demographic/safety data will be provided overall and by BI-RADS of the breast masses to evaluate the consistency of the results across different type of breast masses with respect to BI-RADS. Additional analyses by clinical and/or demographic subgroups may be performed. Confidence intervals will be calculated at a 95% confidence level, unless otherwise specified. Given the nature of the study (i.e., early observational study), no formal statistical hypothesis testing is planned for this study. Baseline subject characteristics will be tabulated. Baseline subject characteristics will comprise the following: subject demographics (gender, age, race, ethnicity), height, weight, Fitzpatrick skin type, menopausal status, cup size, Standard of Care (SOC) imaging (i.e. mammography, Conventional Diagnostic Ultrasound (CDU), magnetic resonance imaging), Probability of Malignancy (POM) and BI-RADS, breast mass location and depth and size, lymph node location and size and depth, breast and lymph node medical/surgical history. For baseline subject characteristics that are continuous variables, the following descriptive statistics will be given: number of observations, mean, standard deviation, minimum, maximum, and median. For subject characteristics that are categorical variables, the frequency and percentage of subjects in each category will be provided. Summary tables will display demographic and baseline characteristics overall and by BI-RADS of the breast masses (3, 4a,

	4b, 4c, 5). Adverse events, serious adverse events, and unanticipated serious adverse device effects will be summarized for the safety analysis population by presenting the number and percentage of subjects in each of these categories overall and by severity.

Table of Contents

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
BI-RADS	Breast Imaging-Reporting and Data System
CDU	Conventional Diagnostic Ultrasound
CRF	Case Report Form
CRO	Clinical Research Organization
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IRB	Institutional Review Board
IUS	Imagio ultrasound using the duplex probe
LOM	Laser Optical Movies
LOM/POM	Likelihood/Probability of Malignancy
MedDRA	Medical Dictionary for Regulatory Activities
NLR	Negative Likelihood Ratio
OA	Opto-Acoustic
OA/US	Refers to the use of the Imagio system (i.e. both IUS and OA function combined + SenoGram)
POM	Probability of Malignancy
QA	Quality Assurance
QAR	Quality Assurance Radiologist
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SOC	Standard of Care
UADE	Unexpected Adverse Device Effect
USADE	Unanticipated Serious Adverse Device Effect

REVISION HISTORY

Version	Date	Summary of Changes
1.0	5/12/2021	*Not applicable, first release.*
2.0	7/14/2021	*Section 2 Study Design and Objectives – clarification to number 1:* This includes doppler and elastography imaging with gray scale only ultrasound probe and ultrasound mode of the OA/US Duplex probe *Section 3 - Removal of inclusion criteria 5* *Section 4.3 Subject Data – Clarification to images required* *Section 4.4 Scan Procedure and Imagio Imaging – Clarification to number 3 – biopsy imaging required for up to 3 subjects and timepoints for biopsy imaging clarified per probe* *Other Administrative Revisions*
3.0	8/17/2021	*Remove exclusion for focal pain* *Administrative Revisions*
4.0	11/10/2021	*Section 4.4 Remove the need for CDU Elastography and Doppler imaging prior to Imagio Elastography and Doppler Imaging. Administrative Revisions*

1. INTRODUCTION

1.1. Background and Significance

Breast cancer is the most common cause of cancer-related death for women [1]. According to the World Health Organization, the highest rate of breast cancer cases was observed in Europe in 2012 [2]. Unfortunately, breast cancer remains difficult to definitively diagnose without performing a biopsy, despite the existence of multiple screening and diagnostic imaging methodologies.

There are multiple reasons for the bias toward performing a biopsy beyond the psychological burden of a suspicious mass in the breast. Breast cancer is a heterogeneous disease with variability in genetic and hormonal influences, which contribute to its varied pathobiology [3]. Consequently, the clinical and radiographic manifestations and histopathology of breast cancer are variable. Furthermore, breast tissue is also heterogeneous, containing fat, glandular and fibrous tissue. The structure of the breast and the nature of breast cancer present a great challenge for imaging technologies to detect cancerous masses reliably and to differentiate them from benign masses.

Seno Medical Instruments, Inc.'s imaging technology attempts to address this challenge by utilizing two very different real-time spatially co-registered and temporally interleaved modalities, opto-acoustic (OA) and diagnostic ultrasound (US), thus providing co-registered and temporally fused functional and anatomical information. This combination has the potential to add diagnostic

information to help the radiologist evaluate breast masses in an efficient and cost-effective manner.
Device Description

1.1.1. Imagio® Imaging System

The Imagio Breast Imaging System (Figure 1A) is a diagnostic functional and morphologic imaging system that combines optoacoustic (OA) imaging with conventional diagnostic ultrasound (US) imaging to aid in diagnostic evaluation of breast lesions. The Imagio Breast Imaging System acquires, processes, and displays co-registered OA and US images to provide structural and functional information about breast abnormalities in real-time. OA/US imaging technology combines the high contrast resolution of optical imaging with the high spatial resolution and deep penetration of ultrasound imaging.

In OA imaging (also known as photoacoustic imaging in the literature), short nanosecond pulses of laser light are delivered into tissue. When that light is absorbed, for instance by hemoglobin molecules found in blood, those molecules undergo thermoelastic expansion and generate acoustic pressure signals that can be detected at the tissue surface using a handheld probe (Figure 1B & 1C). The probe has optical windows that deliver light to tissue and an array of piezoelectric transducers that detect acoustic signals. The received OA signal increases with both the absorption strength and concentration of light-absorbing molecules. Two strong absorbers in tissue are oxygenated and deoxygenated hemoglobin found in blood vessels. By performing OA imaging using two near-infrared optical wavelengths (757 nm and 1064 nm) for which oxygenated and deoxygenated hemoglobin absorb light differently, the Imagio Breast Imaging System can estimate the relative oxygen saturation of hemoglobin in tissue.

A **B** **C** **D**

FIGURE 1. IMAGIO BREAST IMAGING SYSTEM (A); GRAYSCALE U/S PROBE (B); OA/US PROBE (C); EXAMPLE IMAGE AS 6-ON-1 DISPLAY (D)

In addition to OA imaging, the Imagio Breast Imaging System provides conventional ultrasound imaging capabilities such as 2D B-mode (amplitude), color Doppler, power Doppler, and pulsed wave Doppler for blood/fluid flow analysis, user-adjustable image display settings, and the ability to measure and annotate images. The Imagio Breast Imaging System can provide overlaid OA/US images that co-register anatomical US B-mode images with functional OA mapped information in real-time. These images are presented as a 6-on-1 (six-up) display (Figure 1D). The grayscale US image provides morphologic information, whereas the OA/US images provide both morphologic and functional information. The morphologic information provided by OA/US includes: the presence or absence of detectable blood vessels as well as the number, size, shape, orientation, and polymorphism of blood vessels. The functional information provided by OA/US includes the relative amount of hemoglobin and relative oxygen saturation of hemoglobin.

The Imagio Breast Imaging System was determined to be a Class 3B laser product for externally accessible emission from the handheld probe and a Class 4 laser product for internally accessible emission (during device maintenance/service). The Imagio's laser subsystem is comprised of a laser head, laser controller, temperature controller, and footswitch. The laser head generates the pulsed laser emissions, directing them into an optical port which is coupled with a fiber optic cable connecting to the handheld probe. The laser controller provides real time control of laser emission timing and energy levels. The temperature controller regulates the laser head temperature to maintain optimal laser head operation by heating or cooling water that is then pumped through portions of the laser head at a constant flow rate. The foot switch must be engaged by the operator to allow laser light emission. Light is delivered from the fiberoptic cable through two optical windows in the face of the handheld probe to illuminate underlying tissue. During laser light emission, users, subjects and observers are required to wear provided laser safety eyewear meeting appropriate optical density specifications. The ultrasound imaging component of the device is based on conventional, currently existing ultrasound imaging principles. The ultrasound only probe is currently FDA approved as part of 510K K191007.

1.1.2. SenoGram

To assist the users in assessing Breast Imaging-Reporting and Data System (BI-RADS) classification, the Imagio Breast Imaging System is used in parallel with a SenoGram®. The SenoGram is a software tool, using machine learning, that helps the radiologist predict the probability of malignancy (POM) based on a set of reader-assigned feature scores and other relevant data. The purpose of the SenoGram is to help the users more accurately combine their various feature scores into a single overall score (POM). Unlike some of the more commonly used computer-aided diagnosis systems that use medical images as the input, SenoGram input comes from the users. The user enters 5 feature scores for internal ultrasound (IUS), 5 feature scores for OA and 4 other features: subject age, maximum diameter depth to posterior wall of mass, size of lesion, and mammographic BI-RADS density category (where available). IUS above refers to the

internal ultrasound functionality of the Imagio Breast Imaging System (i.e., without OA).When all data has been entered, the user interface graphically displays the SenoGram Likelihood/Probability of malignancy (LOM/POM) on a scale of 0 to 100. If the user assigns correct feature scores, the SenoGram will perform well; if the user assigns incorrect feature scores, the SenoGram will not perform well. The SenoGram classification model was trained using feature scores from the PIONEER Pivotal Readers, including feature scores from the expert reader assigned during the PIONEER Pivotal Study.

1.1.3. Duplex OA/US Probe

The handheld duplex OA/US probe (Figure 1C) comprises a linear 256 element transducer that can be used for conventional diagnostic ultrasound imaging. In addition, the probe contains optics that are designed to transmit the system laser energy, which is uniformly distributed across two optical windows on the face of the probe. This delivers the light safely and uniformly into the tissue, creating OA signals that are then received by the ultrasound transducer in the probe to create co-registered, temporally interleaved and fused functional OA and anatomic diagnostic ultrasound. The OA duplex probe footprint is no greater than 70 mm long by 40 mm wide, with the active ultrasound transducer size being 50 mm long by 4 mm wide.

1.2. Indications for use

The Imagio Breast Imaging System is indicated for use by a trained and qualified healthcare provider for evaluation of palpable and non-palpable breast abnormalities in adult subjects who are referred for a diagnostic imaging breast work-up, following clinical presentation or either screening or diagnostic mammography or other imaging examinations. The ultrasound mode should be initially used in a targeted fashion, to assess any focal area(s) of clinical or imaging concerns. In ultrasound mode, the device can be used to assign a BI-RADS category to either breast tissue or a mass that is causing clinical or imaging concerns. Masses that are classified as BI-RADS categories 3 through 5 can then be assessed using the Opto-Acoustic (OA) mode. In the OA mode, the Imagio Breast Imaging System provides information about the central nidus, boundary and peripheral zones, based on vascularity and blood oxygen saturation of the imaged tissues, to assist in the diagnosis of the benign or malignant mass(es) of interest. For ultrasound BI-RADS 3-5 masses, using the OA features of the mass, allows for improved classification of the mass of interest as compared to ultrasound alone. The OA mode is not indicated for ultrasound BI-RADS 1 and 2 findings. The Imagio Breast Imaging System includes an artificial intelligence (AI) based software function to assist the users in assessing the BI-RADS Classifications. This device is not intended to be used as a replacement for mammographic screening or for definitive pathologic diagnosis.

1.3. Prior Investigations

Early research proof of concept studies provided the company with supporting data to modify and enhance diagnostic performance of the Imagio. Optimization was achieved using the functional characteristics of real-time opto-acoustic imaging performed at 2 wavelengths of Nd:YAG (Neodymium-doped Yttrium Aluminium Garnet) and Alexandrite lasers. Imagio was subsequently

tested on phantoms simulating breast tissue.

1.3.1. Feasibility Study

A feasibility study was conducted using a total of 155 subjects to determine the ability of Imagio Gen 1 to detect benign and malignant features. The feasibility study provided data to further develop an algorithm to identify separate sets of features that characterize benign and malignant disease. The study included subjects with negative diagnostic ultrasound results and positive diagnostic ultrasound, including results of varying histopathology diagnoses, sizes, locations, depths, ages, ethnicities, and races. These subject-specific characteristics were intended to identify any unusual subpopulations. Exclusions for this study on the use of OA included pregnant subjects, males, subjects with obscured views, and subjects on medications that could activate dermatologic conditions (e.g. photosensitive skin conditions).

The feasibility study demonstrated internal and external features representing tumor morphology [4-6]. The reader assigned POM was significantly higher for malignant masses than for benign masses [7]. The specificity was 8% higher for Imagio than for conventional diagnostic ultrasound (CDU) (24% for OA vs 16% for CDU) among those with benign disease, there was >40% relative reduction in the numbers with POM >2% in support of biopsy sparing [8]. Among subjects with benign disease, 54% of BI-RADS 4a subjects and 23% of BI-RADS 4b subjects had an Imagio POM <2% in support of downgrading [9]. There were no adverse events reported.

1.3.2. System Verification Study

A subsequent System Verification Study was performed, enrolling 44 subjects. The study was conducted at South Texas Radiology Imaging Center to evaluate the dual-wavelength performance of the Imagio Gen 1 Breast Imaging System in classifying suspicious breast masses for final tuning of the OA colorization algorithm prior to embarking upon the pilot phase of the PIONEER Study. The objectives of the System Verification Study were met, and further conclusions could be drawn. There were no reported adverse events (AEs).

1.3.3. PIONEER Study

A prospective multi-center trial, the PIONEER Study, was performed with the Imagio Gen 1 enrolling 2105 subjects enrolled across 16 US clinical sites [10]. Subjects were enrolled upon confirmation of having masses classified as BI-RADS 3-5 by CDU evaluation. Images were collected at the clinical sites and sent to an independent Imaging Core Lab, which processed and checked image quality prior to images being processed and read by seven independent readers.

Of the 1,972 subjects in the Safety Population, which included all subjects exposed to an OA scan (including masses that were a quality assurance [QA] review fail), the overall mean age was 49 years (45 years for subjects with benign lesions and 58 years for subjects with malignant (cancer) lesions). The majority of the population was white (n = 1,558; 79%). Seventy-two subjects (<4%) were noted to have more than one mass.

The study had one primary effectiveness endpoint and five secondary effectiveness endpoints, all of which were achieved with statistical significance after accounting for multiplicity testing.

Analyses were conducted on the intent to diagnose the population which consisted of any subject included in the Safety Population with at least one mass that passed QA review and had a valid biopsy (as applicable for BI-RADS 3, 4 and 5). Results were as follows:

- Primary effectiveness endpoint (OA specificity)
  - An overall 14.9% absolute specificity advantage for OA/US vs IUS ($p < 0.0001$)
- Secondary effectiveness endpoint (OA/US sensitivity)
  - OA/US sensitivity was demonstrated to be non-inferior to IUS ($p < 0.0001$), relative to a pre-defined 5% non-inferiority margin
- Secondary effectiveness endpoint (OA/US specificity)
  - OA/US specificity was 43.0% which rejected the pre-planned 40% null hypothesis, supporting the OA/US secondary sensitivity hypothesis (lower bound of 99%, confidence bound >40%)
- Secondary effectiveness endpoint (OA/US sensitivity)
  - OA/US sensitivity was 96.0% which rejected the pre-planned 90% null hypothesis, supporting the OA/US secondary sensitivity hypothesis (lower bound of 99%, confidence bound >94%)
- Secondary effectiveness endpoint (downgrading of BI-RADS in benign masses)
  - OA/US reads downgraded 48.6% of IUS BI-RADS 3 benign masses to BI-RADS 2; a 25% alternative hypothesis was tested relative to no advantage (null hypothesis)
  - OA/US reads upgraded 21.3% of IUS BI-RADS 3 benign masses to 4a+
  - OA/US reads downgraded 29.1% (1,578/5,418) of IUS BI-RADS 4a+ benign masses to a BI-RADS 2 or 3
  - Overall, in benign masses, OA/US downgraded 34.5% of BI-RADS (BI-RADS 4a+ to ≤3, or 3 to 2), in contrast to 6.0% being upgraded, for a net downgrade percentage of 28.5%
- Secondary effectiveness endpoint (upgrading of BI-RADS in malignant masses)
  - OA/US reads resulted in upgrading 47.0% of malignant masses relative to IUS; a 10% alternative hypothesis was to be tested relative to no advantage (null hypothesis). OA reads resulted in downgrading 27.3% of these reads.
  - Thus, for malignant masses, the net gain (using the same denominator) was 19.7% (47.0% - 27.3%).

There were no serious adverse device effects (SADEs), unexpected adverse device effects (UADEs), or deaths reported during the Study. Ten subjects (0.5%) reported 11 AEs that were considered related to the OA procedure.[1] None were serious. All were mild in severity.

[1] 7 events of paraesthesia reported in 7 subjects (0.4%); and 1 event of burns second degree, procedural pain, erythema and skin warm reported in individual subjects (0.1% each).

1.3.4. Reader-02 Study

The Reader-02 Pivotal Study met its primary endpoint and demonstrated that Imagio Breast Imaging Gen 1 System has better specificity than IUS at a fixed sensitivity of 98%. Specificity at a fixed sensitivity of 98% was analyzed using multi-reader multi-case (MRMC) analysis (Obuchowski-Rockette Dorfman-Berbaum-Metz (ORDBM) MRMC 2.51). Mean (average over all readers) fixed specificity (fSp) was found to be higher with statistical significance (two-sided p=0.027) for IUS+OA (47.2%, 95% CI=[35.9%,58.5%]) compared to IUS alone (38.2%, 95% CI= [24.9%, 51.6%]), with a difference in fSp of 9.0% (95%CI=[1.0%, 17.0%]). Thus, IUS+OA achieved the primary endpoint in the Reader-02 Pivotal Study. The decrease (i.e., improvement with IUS+OA compared to IUS alone) in negative likelihood ratio (NLR) could not be established with statistical significance because the observed relative NLR (the ratio in NLR for IUS+OA and IUS) was 0.896 with a 95% CI= (0.693, 1.11) which included 1, indicating that no evidence of a difference in NLR was found. Based on descriptive statistics that do not control type I error and that cannot be generalized outside this particular study, the observed mean positive likelihood ratio (PLR) was 1.959 (95% CI: 1.870, 2.051) for IUS+OA (only as a descriptive result this suggests that averaging over all 15 readers in the study a positive test read (POM>2%) was observed about 2 times more often among cases with cancer, compared to those without cancer), and the mean PLR was reported as 1.548 (95% CI: 1.498, 1.597) for IUS alone (only as a descriptive result this suggests that averaging over all 15 readers in the study a positive test read (POM>2%) was observed about 1.5 times more often among cases with cancer, compared to those without cancer). The descriptive observed relative PLR was 1.281 (95% CI: 1.231, 1.298).

Partial area under the curve (pAUC) was lower in the hierarchical test order than NLR, which failed to achieve significance. Consequently, pAUC results are not part of the claim structure and are reported as descriptive statistics. The mean unscaled pAUC was 0.0244 (95% CI: 0.0230, 0.0258) for IUS+OA and 0.0205 (95% CI: 0.0191, 0.219) for IUS alone, with a difference of 0.0039. All readers had a larger point estimate of pAUC for IUS+OA than for IUS alone.

Of the 480 subjects who contributed masses for reading in the Reader-02 study, 16 (3.3%) experienced at least one AE during the study, of which 6 (1.3%) occurred on the date of the Imagio procedure. Three subjects (0.6%) had at least one Imagio procedure-related AE. There were no AEs that were graded as severe; 14 (2.9%) were considered mild and two (0.4%) were graded moderate. All Imagio procedure-related AEs were considered mild. Two subjects (0.4%) experienced a serious adverse event (SAE), neither of which was Imagio procedure-related; one experienced rupture of a saline breast implant, requiring revision surgery, and one was diagnosed with Stage I non-small cell lung cancer and underwent resection of the affected lobe in the right lung. There were no SADEs or UADEs.

1.3.5. MAESTRO Post-Marketing Study – Europe

Another prospective multi-center study included results of 209 subjects with the Imagio Gen 1 enrolling 215 breast masses classified as BI-RADS 4a or 4b by CDU [11]. Subjects were enrolled at 5 sites between 2015 and 2016. Masses that were classified as BI-RADS 4a or 4b by CDU were

entered into the MAESTRO study, and all were biopsied. The absolute OA/US specificity was 41.1% and the improvement in specificity with OA/US was also 41.1% because, by definition, the CDU findings in any benign mass classified as BI-RADS 4a or 4b would be falsely positive and specificity for CDU will be 0%. The OA/US sensitivity in MAESTRO was 95.5%. The NLR was 0.11. There was one moderate adverse event, a hematoma post biopsy, that was unlikely related to the OA/US procedure.

1.4. Study Motivation and Rationale

This study performs the first clinical evaluation of the Gen 2 Imagio System in a clinical setting to assess image quality with both the IUS ultrasound only probe and the OA/US (duplex probe). This study assesses palpable and non-palpable breast abnormalities in adult subjects who are referred for a diagnostic imaging breast work-up, following clinical presentation, or screening mammography, or diagnostic mammography, or other imaging examinations.

2. STUDY DESIGN AND OBJECTIVES

This clinical investigation is designed to evaluate the images produced by the Gen 2 Imagio System in a clinical setting.

The primary objectives of this study are:

1. Obtain ultrasound only probe images and OA/US (both gray scale and OA) probe images. This includes doppler and elastography imaging with gray scale only ultrasound probe and ultrasound mode of the duplex OA/US probe.
2. Provide breast and lymph node pathology results (except BI-RADS 1, 2 and 3 (as applicable))

3. SUBJECT POPULATION

A maximum of 38 healthy subjects ≥18 years old will be enrolled in the study. The 38 subjects will include the following via Mammo (BI-RADs 1 and 2) or CDU:

- 4 Subjects with BI-RADS 1
- 4 subjects with BI-RADS 2
- 8 subjects with BI-RADS 3
- 8 subjects with BI-RADS 4a
- 4 subjects with BI-RADS 4b
- 4 subjects with BI-RADS 4c
- 6 subjects with BI-RADS 5

3.1. Inclusion/Exclusion Criteria

Inclusion Criteria: Subjects must meet all the following criteria to be included in the study:

1. Has a signed and dated informed consent, prior to initiation of any study-related activities.
2. Is at least 18 years of age.
3. Have been referred for a breast US because of a breast lesion/mass finding via a palpable lump or per standard of care imaging.
4. Is willing and able to comply with protocol-required scans.

Exclusion Criteria: Subjects who meet any of the following criteria will be excluded:

1. Is pregnant or lactating.
2. Has a condition or breast impediment (i.e. insect bites, poison ivy, rash, open wounds, chafing of the skin, scar, tattoos, moles, hematoma, nipple rings, etc.) which could interfere with the intended field of view.
3. Is experiencing photo-toxicity associated with currently taking, or having taken, photosensitizing agents within the previous 72 hours such as some sulfur containing drugs, ampicillin, tetracycline.
4. Is currently undergoing phototherapy.
5. Has a history of any photosensitive disease (e.g., porphyria, lupus erythematosus) or undergoing treatment for a photosensitive disease and is experiencing photosensitivity.
6. Has had an adverse reaction in the past to medical laser procedures, such as laser hair removal or laser tattoo removal.
7. Has had prior benign excisional breast biopsy on breast of interest within the past 18 months.
8. Currently has mastitis.
9. Prisoners

These inclusion and exclusion criteria will be confirmed prior to the start of each scan procedure.

3.2. Informed Consent

Written informed consent will be obtained from every subject. Once consented, the subject will be considered enrolled in the study. Screen failure data will be collected. A subject is considered a screen failure post signing the informed consent.

The informed consent interview will be performed, and each subject will be given personal time and the opportunity to ask questions. The time for decision making prior to giving consent or declining participation will include the time to slowly read and to discuss the consent form. The IUS (ultrasound only probe) and OA/US (duplex probe) imaging procedure will be performed after consent. The subject will receive a copy of the Informed Consent Form for their records.

Upon enrollment, subjects receive a subject number that will be used on all documentation for the subject throughout the study. Subject numbers will be assigned in ascending order, and numbers will not be omitted or reused. The subject number is coupled with the site identification number for

unique identification of each subject.

3.3. Enrollment Failures

Subjects deemed enrollment failures according to the quality assurance review or failure to complete the biopsy evaluations for BI-RADS 3 (as applicable), BI-RADS 4 and 5 will be replaced during the active enrollment period of the study. The following subjects will be considered enrollment failures and will be followed until all AEs are resolved:

- Subjects found to be ineligible after the scan is conducted
- Subjects for whom the study device scans do not pass review (Imaging Core Lab Quality Assurance Radiologist (QAR) review)
- BI-RADS 3 (as applicable) 4 and 5 subjects who have incomplete or unusable biopsy results

4. STUDY PROCEDURE

4.1. Study Device

The study will use the Gen 2 Imagio to generate grayscale ultrasound only (IUS)and OA/US images as applicable depending on the subjects mass or abnormality. The Gen 2 OA/US duplex probe and device is an investigational device in the US.

The Imagio OA/US probe and device will not be used to make any subject clinical decisions during this study.

4.2. Operator Credentials and Training

The Imagio system will be operated only by a trained and qualified healthcare provider.

The Subject, the Imagio Breast Imaging System User, and everyone else in the scan room must always wear Seno approved laser protective eyewear when the Imagio Breast Imaging System is powered on and the laser is enabled

4.3. Subject Data

Data to be collected:

- Gender
- Age
- Race
- Ethnicity
- Fitzpatrick skin type
- Breast and lymph node medical/surgical history

- Height
- Weight
- Inclusion/Exclusion criteria review
- Breast Mass location and depth and size
- Breast Mass feature Scores
- Image Artifact Score
- Lymph node location and size and depth
- Lymph Node Scoring
- Standard of Care (SOC) Imaging POM and BI-RADS
- Imagio IUS POM and BI-RADS using ultrasound only probe and OA/US POM and BI-RADS post SenoGram for breast mass
- IUS ultrasound only probe imaging, duplex OA/US probe imaging (doppler and elastography images as applicable with duplex OA/US probe in ultrasound only mode), and SOC imaging for each breast study mass and study lymph node (as applicable)
- Breast biopsy images for ultrasound only probe and duplex OA/US probe in ultrasound only mode and pre and post biopsy duplex OA/US probe in OA mode images as possible
- Mass and lymph node pathology and surgical report for BI-RADS 3 (as applicable), 4 and 5 masses
- Device Adverse Events/Effects
- Reason for End of Study

4.4. Scan Procedure and Imagio Imaging

Each subject depending on BI-RADS score will have the breast and as applicable the axillary lymph nodes imaged with the ultrasound only probe and the Imagio OA/US duplex probe. The expected duration of a subject's participation would be a total of one visit. The duration of the imaging session will be determined by the subject's availability and may be up to approximately 60 minutes in duration. If there are technical difficulties, the scan procedure may extend beyond this timeframe. In addition, if there are technical issues with the saved images, the subject may be asked to return to repeat the scan procedure.

Imagio imaging will be done per the Scan Protocol for each subject.

1. Elasto and/or Doppler and scan with gray-scale ultrasound only probe as possible
2. Elasto and/or Doppler and Scan with ultrasound portion of the duplex OA/US probe as possible.
3. Biopsy imaging as possible (up to 3 subjects only) with the gray scale only probe and the duplex OA/US probe.

    a. Gray-scale ultrasound only probe and OA/US duplex probe in ultrasound only mode:

i. Pre biopsy.
ii. Needle pre-fire and post fire
iii. Post biopsy

b. Duplex OA/US probe in OA mode

i. Pre biopsy
ii. Post biopsy

4.5. Post-imaging subject evaluation

Immediately after the scan procedure, the subjects will be questioned to assess for:

- Any adverse device event/effect

4.6. Minimization of Bias

Potential for bias during this clinical investigation has been minimized by a well-controlled design, expected conduct under the terms of an approved study protocol, use of specific inclusion and exclusion criteria, careful definitions for study procedures and outcomes and prospectively defined methods of data analysis. No blinding/masking will be conducted.

5. SCHEDULE OF EVENTS

Table 1 shows the schedule of events for this study

Table 1. Schedule of events

Evaluation	Screening/ Enrollment/Baseline Imaging Visit[1] Day 0	Breast and Lymph Node Biopsy Visit (if not done on same day as baseline) May be repeated if issue with initial biopsy *Up to 45 days from baseline	Breast and Lymph Node (as applicable) Surgery *Up to 60 Days from baseline
Informed Consent	X		
Breast/Lymph Node Medical History	X		
I/E Criteria	X		
CDU (including elastography and doppler as applicable) and SOC Imaging	X		
Imagio Imaging (including Elastography and Doppler as applicable) of breast and lymph node (as applicable). This includes biopsy imaging as applicable	X[2]	X	
SOC + Imagio Imaging uploaded to Core Lab	X		
SOC Imaging, Imagio IUS and/or IUS+OA POM and BI-RADS Scoring	X		
Pathology/Surgical Reports as applicable		X	X
Adverse Event/Effect	X		
End of Study	X[3]	X[3]	X[3]

[1]The Screening/Enrollment/ Imaging, and Biopsy Visits may take place on the same date as the discretion of the investigator
[2] BI-RADS 1 and 2 abnormalities will be scanned with the ultrasound only probe and possibly the duplex OA/US probe. BI-RADS 3-5 masses will be scanned with both probes.
[3] Study will end at the final definitive diagnosis of the breast mass which could include confirmation via surgery report

6. IMAGING DATA EVALUATIONS

All imaging results will be managed by an independent Imaging Core Lab, who will perform QA review of de-identified collected images and assembly of the designated image sets. A QA evaluator who is a QAR will perform an image/lesion pairing assessment once the core lab performs the QA of all demographic data, checks image completeness and quality, and resolves all discrepancies.

The QARs will check if lesions/masses scanned with Imagio at the site match the same lesion(s)/ mass(es) seen on all imaging modalities at the site as well as on the image guided biopsy.

The Imaging Core Lab to be used in the study is:

American College of Radiology (ACR)
Center for Research and Innovation
50 South 16th Street
Suite 2800
Philadelphia, PA 19102
United States of America

7. STATISTICAL CONSIDERATIONS

7.1. Statistical design and methods

7.1.1. General Statistical Considerations

Summaries of each study endpoint and other clinical/demographic/safety data will be provided overall and by BI-RADS of the breast masses to evaluate the consistency of the results across different type of breast masses with respect to BI-RADS. Additional analyses by clinical and/or demographic subgroups may be performed. Confidence intervals will be calculated at a 95% confidence level, unless otherwise specified. Given the nature of the study, no formal statistical hypothesis testing is planned for this study.

7.1.2. Demographics, Medical History, and Other Baseline Characteristics

For the full analysis set (FAS) population (defined in section 6.3), baseline subject characteristics will be tabulated. Baseline subject characteristics will comprise the following: subject demographics (gender, age, race, ethnicity), height, weight, Fitzpatrick skin type, menopausal status, cup size, Standard of Care (SOC) imaging (i.e. mammography, CDU, and magnetic resonance imaging) POM and BI-RADS, breast mass location and depth and size, lymph node location and size and depth, breast and lymph node medical/surgical history.

For baseline subject characteristics that are continuous variables, the following descriptive statistics will be given: number of observations, mean, standard deviation, minimum, maximum, and median. For subject characteristics that are categorical variables, the frequency and percentage of subjects in each category will be provided. Summary tables will display demographic and baseline characteristics overall and by BI-RADS of the breast masses (3, 4a, 4b, 4c, 5).

7.1.3. Analysis of Study Endpoints

Breast mass site pathology diagnosis (benign, malignant, high risk) will be summarized by presenting the frequency and percentage of breast masses in each BI-RADs category. A listing of the pathology results (benign specification, malignant specification, high risk specification) will be presented.

Breast mass feature scores

Each of the following breast mass feature score data will be summarized by presenting the mean, standard deviation, number of observations, median, quartiles, minimum, and maximum:

- For IUS:
  - External peripheral zone score (range 0-5)
  - External capsular/boundary zone score (range 0-6)
  - Internal shape score (range 0-5)
  - Internal sound transmission score (range 0-5)
  - Internal echotexture score (range 0-5)
- For OA/US:
  - External peripheral zone radiating vessel score (range 0-5)
  - External capsular/boundary zone vessel score (range 0-6)
  - Internal vessel score (range 0-5)
  - Internal deoxygenated blush score (range 0-5)
  - Internal total hemoglobin score (range 0-5)

3. Imagio/OA/US POM and BI-RADS for breast using SenoGram

Investigator POM for breast using SenoGram will be summarized by presenting the mean, standard deviation, number of observations, median, quartiles, minimum, and maximum. Investigator BI-RADS for breast using SenoGram will be summarized by presenting the frequency and percentage of breast masses in each BI-RADS category. SenoGram LOM for breast will also be summarized by presenting the mean, standard deviation, number of observations, median, quartiles, minimum, and maximum.

Lymph node scoring

Lymph node scoring (shape score, cortical thickness score, margin score, hilar compression-displacement score, and size) will be summarized by presenting the frequency and percentage of lymph nodes in each category. The total score will be summarized by presenting the mean, standard deviation, number of observations, median, quartiles, minimum, and maximum. Imagio IUS classification of lymph node will be summarized by presenting the frequency and percentage of lymph nodes in each category.

Lymph node site pathology (histopathology and cytopathology) results will be summarized by presenting the frequency and percentage of lymph nodes in each histopathology (positive for malignancy, negative for malignancy, indeterminate, extracapsular extension) and cytopathology (positive malignancy, negative for malignancy, indeterminate, other) category. A listing of pathology results will also be presented.

7.1.4. Safety Analysis

Adverse events (AE), serious adverse events (SAE), and unanticipated adverse device effects (UADE) will be summarized for the safety analysis population by presenting the number and

percentage of subjects in each of these categories overall and by severity and by the Medical Dictionary for Regulatory Authorities (MedDRA) System Organ Class / Preferred Term. A similar summary will also be provided for device-related and/or procedure-related AEs, SAEs, and UADEs. The number and percentage of subjects who withdrew from the study due to an AE will be presented overall and by System Organ Class and Preferred Term.

A listing of all AEs will be provided that includes details such as AE term (System Organ Class and Preferred Term), days from procedure, seriousness, relatedness to procedure and device, severity, action taken, outcome.

7.2. Sample size

A maximum of 38 healthy subjects ≥18 years old will be enrolled in the study. The 38 subjects will include the following:

- 4 subjects with BI-RADS 1- can be recruited from BI-RADS 0. (Ex. Mammographic asymmetry caused by normal breast tissue, breast pain, etc.)
- 4 subjects with BI-RADS 2- can be recruited from BI-RADS 0. (Ex. palpable lipoma, simple cyst, ductasia, angioma, etc.)
- 8 subjects with BI-RADS 3
- 8 subjects with BI-RADS 4a
- 4 subjects with BI-RADS 4b
- 4 subjects with BI-RADS 4c
- 6 subjects with BI-RADS 5

Given the nature of the study no formal hypothesis testing is planned, and formal power and sample size calculations were not performed. A sample size of 38 subjects is deemed adequate to provide a cross section of breast masses/lesions and lymph nodes.

7.3. Analysis populations

7.3.1. Full Analysis Set (FAS) Population

The FAS population will be defined as all consented subjects who completed screening/enrollment and had a successful Imagio imaging procedure. The FAS population will be used as the secondary analysis population for the study endpoints.

7.3.2. Per-protocol (PP) Population

The PP population will be defined as all FAS subjects described above who meet the study eligibility criteria and have no other major protocol violations. The PP population will be used for primary analysis of the study endpoints.

Within the PP population, summaries of each study endpoint will be provided overall and by

subgroups based on BI-RADS (3, 4a, 4b, 4c, 5) of the breast masses.

7.3.3. Safety Population

The safety population will be defined as all subjects in whom the Imagio imaging procedure was attempted. This analysis population is the primary population for all safety analyses.

7.4. Missing data

Given the nature of the study, analyses will be performed for observed (available) cases only. No imputation of missing data will be performed.

7.5. Interim analysis

Interim analyses are not planned for this study.

7.6. Statistical deviations

Any post-hoc, or unplanned, analyses not identified in the protocol will be clearly identified in the study report.

8. RISKS AND BENEFITS

8.1. Potential Risks

Subjects will be positioned in an identical manner to that of undergoing diagnostic ultrasound. The subject will be asked to wear protective eyewear once settled into position. The duplex probe and protective eyewear are designed to be wiped down with cleaner/disinfectant after every use.

The Imagio laser classification falls under the Class IIIB laser designation. During OA imaging, the laser shutter is opened, and the laser delivers near infrared pulses to the subject's target location through a fiber-optic beam delivery system built-into the duplex OA/US probe. Direct beam laser illumination from the OA laser can potentially cause damage to the unprotected eye. The risk to the eyes is mitigated using protective eyewear specifically designed for protection against laser illumination in the near infrared spectral range. This protective eyewear is required to be worn by operators, subjects, and any other personnel in the examination room.

ANSI Z136.1-2014, *American National Standard for Safe Use of Lasers*, published by the Laser Institute of America defines the maximum permissible exposure safety threshold for skin exposure as 20 mJ/cm^2 for the case of multiple near infrared laser wavelengths and low repetition rate nanosecond laser pulses. The repetition rate of the laser pulses is limited to assure that the laser-induced pressure and heat dissipates from the illuminated volume during the time between two consecutive laser pulses. OA uses a pulse repetition rate of up to 20 pulses per second with laser fluence less than 20 mJ/cm^2 at any point.

The Imagio Imaging System has been evaluated for acoustic output, biocompatibility, cleaning, and disinfection. The system has been found to conform with thermal, electrical, electromagnetic, and mechanical safety and has been found to conform to applicable medical device safety standards.

Potential risk of data disclosure will be addressed by proper data management procedures (e.g. immediate study number assignments, de-identification of data, and with the subject number kept in a place of physical security).

Possibility of physical injury is primarily associated with violation of procedures of safe use of lasers. Subjects and all personnel will wear protective eyewear to minimize the risks to the eyes. The risk of skin injury is minimal with the use of safe levels of laser illumination.

8.2. Blinding

Subjects will not be blinded to best practice study procedures but will not have access to the Imagio OA/US evaluation result.

The site investigators will have access to all subject data including the Imagio OA/US images and will assess and document the results of the OA/US study by completing the electronic data entry form (electronic case report form (eCRF)).

The site investigators will have access to the background clinical information and to the subsequent histopathology report.

8.3. Anticipated Adverse Device Effects

List of potential risks and discomforts:

- Tingling or warmth of the skin during the Imagio scan (0.4% or a 1 in 269 chance) that resolve at completion of the scan
- Exposure to the acoustic output
- Contamination due to insufficient cleaning
- Phototoxicity

8.4. Potential Benefits

There are no direct benefits to subjects participating in this study. However, evaluating the opto-acoustic image quality of the Gen 2 System in a clinical setting may contribute to improved diagnostic information. We hope that improved Imagio OA/US image quality will contribute to more accurate diagnosis of breast masses in future clinical use.

The Gen 2 System will not be used to make any clinical decisions.

8.5. Risk/Benefit Rationale

Seno Medical Instruments, Inc. believes that any potential risk presented by this investigation has been minimized and that adequate testing, safeguards, and safety monitoring have been incorporated into the design of the product and it's accessories to further mitigate subject risk and exposure to potentially harmful laser light.

Other risks associated with the device are based on nonclinical laboratory testing and have been mitigated per ISO 14971. The device is a Class 3B laser product for external exposures (i.e., through the imaging probe) and Class 4 for internal exposures (i.e., during maintenance). Laser exposure is below known safety limits for skin and eye (when wearing appropriate laser safety eyewear).Therefore, this observational study, done to assess images is felt to be safe and aligned with the previously approved P200003 Imagio Breast Imaging System.

9. MEDICAL MONITOR

The medical monitor is a physician who will provide safety oversight for the investigation. Responsibilities of the medical monitor include, but are not limited to:

- Provide medical and scientific input to clinical data and subject medical safety data
- Maintain ongoing assessment of the safety profile of the investigational device during the investigation
- Provide medical surveillance and evaluation of Serious Adverse Events (SAEs) and unanticipated adverse device effects (UADEs).

The medical monitor for this study is:
A. Thomas Stavros, MD
Chief Medical Officer
Seno Medical Instruments, Inc.
8023 Vantage Drive, Suite 1000
San Antonio, TX 78230, USA
Phone: 210-615-6501

10.ADVERSE EVENTS

10.1. Adverse Event Assessments

Criteria outlined in this section will be used to evaluate each subject during their participation in the study.

10.1.1. Assessment of Severity

10.1.1.1. Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a subject. This means any clinically adverse sign, symptom, syndrome, or illness that occurs or worsens during the visit, regardless of causality, that is not otherwise being measured in the study. It includes any written, electronic, or oral communication with regard to a company sponsored clinical investigation that alleges deficiencies related to the investigational device resulting in an undesirable occurrence or untoward deviation in health away from the subject's baseline. Only device related adverse events will be collected during this study.

Each AE will be evaluated for its seriousness, severity, and association with the Imagio. Adverse events will be followed until resolution.

10.1.1.2. Serious Adverse Event (SAE)

The severity of AEs will be assessed according to the 21 CFR 812 SAE definition. A device-related SAE will be defined as an SAE in which the Imagio device relationship cannot be ruled out. An AE that meets any of the following criteria will be considered an SAE for protocol purposes:
1. Led to death
2. Led to a serious deterioration in the health of the subject that either:

- Resulted in a life-threatening illness or injury
- Resulted in permanent impairment of a body structure or a body function
- Required in-subject hospitalization or prolongation of an existing hospitalization
- Resulted in medical or surgical intervention to prevent permanent impairment of a body structure or a body function.

Additionally, an AE that meets either of the following criteria will be considered reportable as a serious event to meet SAE regulatory reporting criteria:

3. Resulted in persistent or significant disability or incapacity; or
4. Important AEs that are not immediately life-threatening or do not result in death or hospitalization but, based on the physician's medical judgment, may jeopardize the subject, or may require intervention to prevent one of the other outcomes listed above.

10.1.1.3. Unanticipated Adverse Device Effect (UADE)

An UADE is "any unanticipated 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, or any other unanticipated serious problem associated with a device that relates to the rights, safety, and welfare of subjects". An observer in this study must report any UADE effect to the Institutional Review Board (IRB) within 24 hours of learning of the occurrence. Seno will then report the UADE effect, as required by the Food and Drug Administration (FDA) guidelines.

10.1.2. Evaluation of Severity

The severity of AEs will be assessed according to the following definitions:

Mild: The AE is noticeable to the subject but does not interfere with routine activity. The AE does not require any action in connection with the study.

Moderate: The AE interferes with the subject's routine activity but responds to symptomatic therapy or rest. The AE may require action concerning the study.

Severe: The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. The AE may require action concerning the study.

Life-Threatening: The AE is of a nature that the subject is at immediate risk of death, even if not related to study activities. The AE may require action concerning the study.

10.1.3. Evaluation of AE Relationship to Device

The relationship between an AE and the study device (investigational device) will be determined by the Principal Investigator based on his or her clinical judgment and the following definitions:

Table 2. Evaluation of AE Relationship to Device

Likely related	AEs with clear temporal and/or spatial relationship to Imagio device, with no clear alternative underlying cause.
Probably related	AEs with reasonable temporal and/or spatial relationship to Imagio device that cannot be readily explained by the subject's clinical state or other agents/therapies.
Possibly related	AEs with fair temporal and/or spatial relationship to Imagio device, with no clear alternative underlying cause.
Not related	AEs that do NOT follow a temporal and/or spatial relationship to the Imagio device and can be readily explained by an alternative cause, such as an accidental injury or expected progression of an underlying or concomitant disease.

10.1.4. Reporting of Adverse Events

The Investigator will determine only device related adverse device effects. AEs will be reported on the AE CRF page, and each will be classified according to the aforementioned criteria. Adverse event reporting for each subject will begin upon the subject entering the Imagio scan room and continue through the Imagio procedure and subjects exit of the Imagio scan room. Any pre-existing conditions that are detected as part of the enrollment procedures must be reported in the medical history and not as an AE unless they worsen as a result of the Imagio procedure. Regulatory reporting standards will be followed for qualifying serious or unexpected events as per 21 CFR 812.

10.1.5. Reporting Serious Device Adverse Event (SAE) and Unanticipated Serious Adverse Device Effect (USADE) Recording

The Investigator shall complete the SAE/USADE form. If the event has been classified as a SAE/USADE, details of medical history and concomitant medications (as applicable) will accompany the form. The Investigator must report the SAEs and USADEs promptly within 24 hours of the event occurring.

All SAE and USADE data collection forms will be collected and placed within regulatory binder and submitted to the IRB as required per IRB guidelines.

10.1.6. Reporting Adverse Event (AE) and Unanticipated Adverse Device Effect (UADE) Recording

The same guidelines listed above under 9.1.4 apply to UADEs. Device related AEs will be recorded within the adverse event data form.

10.2. Removal of Subjects from the Study

A subject may be withdrawn from the study for any of the following reasons:

- A serious or intolerable adverse event/effect or medical event occurs that in the investigator's opinion would preclude subject from continuing with the study
- Seno terminates the study
- Subject enters the study in violation of the protocol, and it is subsequently discovered, and it is not in the subjects best interest to continue
- Subject deviates from the protocol during the study and it is not in the best interest of the subject or sponsor for subject to continue
- Pregnancy
- Subject withdrawal of consent or subject requests to be withdrawn

11. PROTOCOL AND PROTOCOL AMENDMENT REVIEW AND APPROVAL

Seno and the IRB will be required to approve the initial study protocol and applicable documents. Any amendment to the study protocol must be approved by the sponsor. A protocol amendment may not be implemented until after it has been approved by the IRB unless immediate implementation of the change is necessary for subject safety. In this case, the protocol change must be documented in an amendment.

11.1. IRB Committee

The protocol and the informed consent form must have the approval of a properly constituted IRB Committee responsible for approving clinical studies. The signed IRB Committee approval letter

must specify the date of protocol and informed consent form approval and identify the documents approved including the investigator's name, the protocol version, date, and title. Any subject materials or advertisements used to recruit subjects should also be reviewed and approved by the IRB Committee.

12. MONITORING

Monitoring of the study will be performed in accordance with FDA guidelines and Good Clinical Practice.

Monitoring will be performed by Seno or Seno's representatives on a periodic basis. During monitoring sessions, data recorded and entered directly into the eCRF will be considered source. Any data recorded outside of the eCRF will be source and the eCRF will be verified against it for accuracy and completeness. The monitor should have direct access to source data for verification purposes. Such verification is essential to quality control.

Delegation of study tasks will be the responsibility of the Principal Investigator and recorded on the Study Delegation Log.

13. DEVICE STORAGE AND ACCOUNTABILITY

Seno will ship Imagio devices to the participating site. Seno personnel will unpack and install the study device as well as conduct and document operator training for study personnel.

The Principal Investigator and designated site personnel will be responsible for ensuring that only authorized personnel have access to the study device for study purposes. Accountability of the devices to be used in the study will be maintained.

14. QUALITY ASSURANCE

The sponsor, or the sponsor's representative, may conduct internal audits. Audits may include, but are not limited to, device supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. Source documents would include any records of the first-time data is recorded during the study beyond the data recorded directly into the eCRF plus the study images. The IRB may request access to source data during the conduct of the study.

15. ETHICAL CONDUCT

This study will be conducted in accordance with the ethical principles outlined within FDA and Good Clinical Practice (GCP) regulations as well as local IRB requirements.

16. PROTOCOL DEVIATIONS

Protocol deviations may occur in two ways:

- Deviations from the protocol, contrary to protocol specifications (i.e., deviations from the protocol procedures, eligibility, instructions for use, visit windows, etc.).

- Deviations affecting the endpoint outcome not previously specified in the protocol (i.e., deviation that had not been previously considered in the protocol or eligibility criteria, but having a clear impact on the primary outcome measure)

The investigator is not allowed to deviate from this protocol except as specified in FDA regulations. The Investigator should not implement any deviation from or changes to the protocol without approval by Seno and prior review and documented approval/favorable opinion from the IRB of a protocol amendment, except where necessary to eliminate immediate hazards to study subjects, or when the changes involve only logistical or administrative aspects of the study (e.g., change in monitors, change of telephone numbers). While every effort should be made to avoid protocol deviations, should a deviation be discovered, Seno must be informed immediately.

Deviations will be documented in the eCRF. The Investigators will also adhere to procedures for reporting investigation deviations to their IRB committee in accordance with their specific reporting policies and procedures.

Prior to final database lock, all protocol deviations will be reviewed and categorized as major (i.e., those that affect measurement or interpretation of the primary endpoint or related to safety) or minor (those not affecting the primary endpoint). The presence of major deviation may exclude subjects from the Intention to Diagnose (ITD) analysis population upon review by the Protocol Deviation Committee.

16.1 Corrective and Preventative Actions

Seno Medical and her representatives will evaluate protocol deviations during monitoring visits. Individual event corrective and preventive actions may be recommended at that time.

17. INVESTIGATOR DISQUALIFICATION CRITERIA

Seno Medical reserves the right to terminate an investigator/investigational site for any of the following reasons:

- Failure to secure subject informed consent including protection of personal data prior to enrollment.
- Failure to report unanticipated adverse device effects within 24 hours of discovery (to Seno Medical) and to the IRB committee within its required reporting time after learning of the event.
- Failure to report serious adverse device effects within 24 hours of discovery.
- Repeated investigational plan deviations.
- Repeated failure to appropriately complete case report forms.
- Failure to enroll an adequate number of subjects.

- Loss of or unaccounted for investigational product inventory.

18. SUBJECT CONFIDENTIALITY

Confidentiality will be maintained in accordance with FDA and IRB regulations. Subject names must not be revealed to the sponsor or sponsor's representatives. Only the subject identifier (number) will be recorded in the eCRF and if the subject's name appears on any other document, it must be redacted and replaced with the subject identifier before a copy of the document is supplied to the sponsor or sponsor's representatives. Study findings stored on a computer will be stored in accordance with local data protection laws. In the event of inadvertent communication of such information, immediate steps to redact the information from all study files will be implemented, with appropriate documentation in the subject study file.

19. CASE REPORT FORMS AND STUDY RECORDS

Data will be recorded using an electronic data capture (EDC) system, according to FDA regulations. The database is considered validated when the expected results are the same as the actual results, and the end users verify that the database performs according to the requirements.

The data will include collecting images plus the Laser Optical Movie (LOM) to be sent to Seno Medical Instruments, Inc.

The validation report, design procedures, testing results, source code, and test eCRF cases are filed in the central files. Database maintenance will be provided throughout the study, as well as user support and administration (access and site user rights set-up, removal, etc.).

The Medical Dictionary for Regulatory Authorities (MedDRA) will be used to code adverse device effects.

The investigator is responsible for maintaining adequate and accurate medical records from which information will be transferred into the study database.

20. DATA MANAGEMENT

A detailed plan for the data management activities will be prepared. The structure of the database will be based on the eCRFs and validated according to the Data Validation Plan, prepared by the Clinical Research Organization (CRO). Discrepancies revealed by data validation will be delivered for resolution with Quality Control Reports. The clean database will be locked after all detected discrepancies have been solved and the database has been updated accordingly. Only authorized and well-documented updates are possible after the database lock. The data will be secured by daily back-ups to the CRO's server (details are included in the Data Management Plan).

21. RETENTION OF DATA

All records must be retained by Seno or Seno's representatives for a period of two years after the latter of the following two dates: the date the study is closed or terminated or the date of the last approval of a marketing application or when records are no longer needed to support a regulatory submission. All study records may be relevant to regulatory inspection.

If the Seno Representative in charge of the study retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility.

22. FINANCIAL DISCLOSURE

Investigators will be asked to provide financial disclosure prior to authorization to begin the study as well as after the study is completed. Investigators will also be expected to share any situations which could introduce specific bias.

23. PUBLICATIONS

The results of the study are the property of Seno Medical Instruments, Inc. All publications (manuscripts, abstracts, or other modes of presentation) must be submitted at a time determined by Seno Medical Instruments, Inc. and must be reviewed and approved in writing by Seno Medical Instruments, Inc., in advance of submission. Co-authorship with any Seno Medical Instruments, Inc. personnel will be discussed and mutually agreed upon before submission of a manuscript to a publisher. Seno Medical will prepare a final study report following the conclusion of the study.

24. LITERATURE CITED

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