SENTINUS

SENTINEL LYMPH NODE IDENTIFICATION WITH CONTRAST ENHANCED ULTRASOUND IN BREAST CANCER

Trial Title: **SENTINUS: Technical feasibility and diagnostic accuracy of intradermal microbubbles and contrast enhanced ultrasound to identify sentinel lymph node metastases in breast cancer patients following training and mentorship of imaging specialists**

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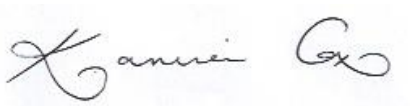
Chief Investigator Signature: 

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1. KEY TRIAL CONTACTS

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2. SYNOPSIS

Trial Title	SENTINUS: Technical feasibility and diagnostic accuracy of intradermal microbubbles and contrast enhanced ultrasound to identify sentinel lymph node metastases in breast cancer patients following training and mentorship of imaging specialists	
Internal ref. no. (or short title)	SENTINUS/ MTW_2020_KC01	
Clinical Phase	II	
Trial Design	Prospective multicentre pilot study incorporating training and mentorship	
Setting	Breast Units in England	
Patients	Women newly diagnosed with early breast cancer with a normal B-mode axillary ultrasound and surgery planned as their primary treatment.	
Planned Sample Size	250 patients from up to 5 Breast Units	
Participating imaging specialists	10 from up to 5 breast units.	
Follow up duration	Surgery will mark the end of the patient's involvement with the trial.	
Planned Trial Period	24 months	
Objectives and outcomes		
Technical Feasibility	To determine whether experienced imaging specialists in up to 5 UK Breast Centres can be trained to consistently identify, core biopsy and clip mark axillary SLN in patients with breast cancer	Technical feasibility will be assessed by 75% of imaging specialists achieving; >85% visualization of tumour draining axillary SLN, >80% successful core biopsy (LN tissue retrieved) rate and >80% concordance with SLN identified surgically with SLN excision.
Diagnostic Performance	To determine the overall diagnostic accuracy of a CEUS SLN core biopsy as a test to identify SLN metastases as compared to the reference standard of axillary surgery.	Diagnostic performance will be assessed by calculating the overall pooled sensitivity, specificity, positive predictive value, negative predictive value of CEUS SLN core biopsy as a test to identify SLN metastases as compared to axillary surgery and the prevalence of LN metastases. An overall sensitivity >50% will be considered acceptable.
Formulation, Dose, Route of Administration	Using an aseptic technique, 1% lignocaine is injected subcutaneously into the peri-areolar upper outer quadrant region. The contrast agent (Sonovue) is reconstituted as per the SmPC and up to 1ml is injected intra-dermally at the site of the local anaesthetic. The breast is gently massaged to encourage the contrast to be taken up by the lymphatics. The axilla is scanned and the contrast software package used on the ultrasound machine allows visualisation of the contrast agent into the axilla. The first draining lymph node is highlighted and biopsied using a 14G conventional core biopsy needle +/- FNA. A marker clip is placed into the lymph node to identify which node has been biopsied. Patients will receive a standard after care leaflet about axillary nodal examination and biopsy.	

3. ABBREVIATIONS

CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
FNA	Fine needle aspiration
GCP	Good Clinical Practice
GP	General Practitioner
ICF	Informed Consent Form
MDT	Multi-Disciplinary Team
MRI	Magnetic resonance imaging
NACT	Neo-adjuvant chemotherapy
NHS	National Health Service
NRES	National Research Ethics Service
PI	Principal Investigator
PIS	Participant/ Patient Information Sheet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGF	Research Governance Framework
RSI	Reference safety information
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
TMF	Trial Master File
USS	Ultrasound

4. BACKGROUND AND RATIONALE

In the UK, excision of tumour draining sentinel lymph nodes is the standard axillary staging procedure for patients with invasive breast cancer and a normal B-mode axillary ultrasound/ benign biopsy of morphologically indeterminate lymph nodes (1). Despite an acknowledged false negative rate <10% (2), trial evidence has shown that substituting the removal of all axillary lymph nodes (axillary lymph node dissection) with sentinel node excision does not negatively impact on overall survival and appears to have little effect on locoregional recurrence (3). The associated morbidities of sentinel node excision are much lower than axillary lymph node dissection, but it remains a surgical procedure performed under general anaesthetic with recognized immediate complications such as infection (11%) and long term problems with sensory loss (11%) and arm lymphoedema (5%) at 12 months (2). The identification of sentinel nodes intra-operatively is currently reliant upon the dual tracer technique using an injection of radioisotope and blue dye to maintain a low false negative rate (6%) (4). The blue dye carries a 0.9% risk of allergic reaction/ anaphylaxis (5) and the procurement of medical grade isotopes is logistically challenging. Newer intra-operative tracers such as superparamagnetic iron oxide particles also have recognized problems including persistent brown staining of the breast (6).

Over the last decade, an innovative system has been developed which utilizes intradermally injected microbubbles and contrast enhanced ultrasound (CEUS) to dynamically image breast lymphatics and follow the vessels to sentinel nodes. Under direct vision, the sentinel node can then be biopsied in the breast clinic. Adapted from a swine melanoma model (7), it was initially described in 2009 by Sever at al at Maidstone, Kent (8) and Omoto et al in Japan (9). Sentinel lymph nodes identified with CEUS correlate well with those identified using standard intra-operative tracers (8). Other academic groups in Europe, Asia and the USA have trialled the procedure indicating that it is a potentially straightforward technique for experienced imaging specialists (10-16). The whole procedural time is 15-30 minutes (17) it is safe and well tolerated by patients (18) and can be performed using ultrasound equipment in widespread clinical use.

B-mode axillary ultrasound, as the current standard of care, usefully recognizes approximately 50% of metastatic LN (19) and evidence indicates that a subsequent CEUS sentinel node core biopsy can identify a further 50% of metastatic axillary lymph nodes (20), thus enhancing the overall diagnostic performance of pre-operative ultrasound. Despite only having a 50% sensitivity, the CEUS sentinel node core biopsy has a high negative predictive value (87%) and any lymph node metastases that are undetected are likely to be low volume and consequently of questionable clinical significance. This was shown in a large (1361 patients) prospective dataset from Maidstone Hospital, where less than 2% of patients with a normal B-mode axillary ultrasound and a benign CEUS sentinel core biopsy had 2 or more axillary LN macrometastases found at the end of surgical treatment. The majority of false negative cases had isolated tumour cells (ITC), micrometastases or a single lymph node macrometastasis with or without ITC/ micrometastases (10). These results were not just confined to patients with favourable tumour characteristics and also included those with large (>50mm) and multifocal cancers (10).

Quantifying axillary lymph node metastases in patients with invasive breast cancer can still be used to guide adjuvant treatment decisions but the loss of this information with the avoidance of axillary surgery may have little clinical impact. Anatomic (surgical) staging of breast cancer is becoming less relevant as clinicians capitalize on the gains made with new genomic and molecular assays as well as updated algorithms to predict response to adjuvant treatment. In the USA, the limitations of anatomical staging

have been acknowledged by the American Joint Committee on Cancer (AJCC) Expert Panels and this has led to the addition of oestrogen receptor (ER) status, Her2 status, grade and molecular characteristics into the 8th Edition Revision published in 2016 and set for implementation in 2019 (21). This change was mainly brought about by the development of new staging systems such as Bioscore that incorporate treatment amenable biologic factors (22).

The concept and practice of surgically removing all malignant axillary lymph nodes to achieve local control and improve survival have also been challenged by the long-term results of a randomized controlled trial (23/24). In the American College of Surgeons Oncology Group Z011 Trial, patients with tumours under 5cm having breast-conserving surgery and whole breast radiotherapy with sentinel node metastases found after sentinel node excision were randomized to a completion axillary lymph node dissection or adjuvant treatment only. At 5 and 10 years, the overall survival, disease free survival and local recurrence rate in the axilla was low with no difference between the groups despite the fact that 27.3% of patients in the axillary lymph node dissection arm arm had further lymph node metastases retrieved at the second operation (23/24).

The findings of the Z011 trial imply that modern adjuvant therapy plays an equally important role in treating axillary lymph node metastases. Patients with high volume axillary lymph node disease should still be identified early on in the pathway and may benefit from surgical tumour de-bulking with lymph node dissection. However, for those with normal appearing lymph nodes on ultrasound and a benign CEUS sentinel node core biopsy, the evidence suggests that it may be a reasonable option to omit axillary surgery altogether without compromising oncological outcomes.

Undoubtedly, the CEUS sentinel node core biopsy is a highly technical skill based procedure, but it should be within the competencies of experienced breast imaging specialists. The technique has 2 distinct components, namely sentinel node identification and sentinel node core biopsy plus marker clip placement. When the individual performance of 7 radiologists at a single institution was examined, the percentage of procedures with successful visualization ranged from 72.8% to 97.5% and the percentage of procedures with a successful core biopsy (LN tissue retrieved) ranged from 71.2% to 99.6% (10). This variation is likely to be due to a lack of training and mentorship but needs to be investigated before wide scale adoption of the procedure in the UK.

5. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Objectives	Outcome Measures/Endpoints
Primary Objective 1 To determine whether experienced imaging specialists in up to 5 UK Breast Centres can be trained to consistently identify, core biopsy and clip mark axillary SLN in patients with breast cancer.	Technical feasibility will be assessed by 75% of imaging specialists achieving; >85% visualization of tumour draining axillary SLN, >80% successful core biopsy (LN tissue retrieved) rate and >80% concordance with SLN identified surgically with SLN excision.
Primary Objective 2 To determine the overall diagnostic accuracy of a CEUS SLN core biopsy as a test to identify SLN metastases as compared to the reference standard of axillary surgery.	Diagnostic performance will be assessed by calculating the overall pooled sensitivity, specificity, positive predictive value, negative predictive value of CEUS SLN core biopsy as a test to identify SLN metastases as compared to axillary surgery and the prevalence of LN metastases. An overall sensitivity >50% will be considered acceptable.
To establish the time taken to perform CEUS SLN core biopsy procedure. To assess the volume of axillary disease. To determine the level of complications (bleeding, infections and pain/sensory disturbances) To assess patient satisfaction of the techniques. To conduct a prospective audit of each unit's detection rate of LN metastases with grey-scale axillary ultrasound.	• Time taken to perform each CEUS SLN core biopsy procedure. • Total volume of axillary disease at the end of primary surgical treatment for each patient • Bleeding complications • Infective complications • Pain/ sensory disturbance • Patient satisfaction • Detection rate of LN metastases with grey-scale axillary ultrasound.

6. TRIAL DESIGN

Phase II prospective multicentre pilot study incorporating training and mentorship of imaging specialists.

Imaging specialists will be recruited from up to 5 Breast Cancer Units within the UK. Three units will have prior experience of using intradermal microbubbles and CEUS to identify and biopsy sentinel lymph nodes in patients with early breast cancer and 2 units will be naïve to the technique. Each unit will put forward imaging specialists to take part in the study (10 imaging specialists in total).

For those units without prior experience of intradermal microbubbles, if necessary, their existing ultrasound machines will be upgraded to allow contrast studies.

Participating imaging specialists will attend a training session at either Maidstone Hospital, Kent, St James's Hospital, Leeds or Guy's Hospital, London. They will have access to video tutorials and written information, which will also be accessible on the SENTINUS Trial website. Dr Jenny Weeks and Dr Nisha Sharma will provide mentorship either by telephone or site visits if necessary. Participating imaging specialists will not receive expenses but refreshments and lunch will be provided during the all-day training session.

During the trial period, each unit can also audit their standard of care diagnostic pathway time from breast cancer diagnosis to the patient knowing their axillary lymph node status (malignant/ not malignant) and compare it with the SENTINUS pathway.

7. PATIENT IDENTIFICATION

7.1. Trial Patients

Following discussion at the breast cancer MDT, female patients aged over 18 years with early invasive carcinoma of the breast with a normal B-mode axillary ultrasound/ benign biopsy of indeterminate lymph nodes and planned primary surgical treatment will be approached to take part in this study. The 5 units will aim to recruit 250 patients) with each of the 10 participating imaging specialists performing 25 procedures. Participating patients can claim a contribution of £30 towards their travel expenses.

7.2. Inclusion Criteria

- Newly diagnosed early invasive carcinoma of the breast with a normal B-mode axillary ultrasound or benign biopsy of indeterminate lymph nodes.
- Surgery as first planned treatment.
- Participant is willing and able to give informed consent for participation in the trial.
- Female, aged 18 years or above.
- In the Investigator's opinion, adhering to the trial recommendations and governance.

7.3. Exclusion Criteria

The participant may not enter the trial if **ANY** of the following apply:

- Previous ipsilateral axillary surgery or ipsilateral breast cancer surgery/ radiotherapy.
- Female participant who is pregnant, lactating or planning pregnancy during the course of the trial.
- Contraindication to contrast.
- Patient cannot provide consent.
- Inflammatory or locally advanced breast cancer.
- Metastatic breast cancer.
- Inability to raise ipsilateral arm above head.
- Multiple medical co-morbidities (ASA 4 or above).

8. TRIAL PROCEDURES

8.1. Recruitment

Eligible patients will be identified through the breast multi-disciplinary team diagnostic (MDT) meetings. The patient will be approached at the subsequent surgical clinic visit. The trial will be discussed in detail and the patient provided with a copy of the Patient Information Sheet. Patients will be offered sufficient time to consider the trial, allowing time for discussion with family/friends/GP. The patient will be given the opportunity to ask questions and to be satisfied with the responses prior to written consent being given, at least 24 hours after the initial approach along with suitable and approved alternative treatment choices. Written informed consent will be obtained by the clinical investigators or nominated individual at the patient's subsequent clinic/radiological visit as per the delegation log in accordance with good clinical practice (GCP) guidelines.

8.2. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as she wishes to consider the information, and the opportunity to question the Investigator, the GP or other independent parties to decide whether they will participate in the trial. Written Informed Consent will be obtained by means of the participant dated signature and dated signature of the person who obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. This must be documented and approved by the Chief Investigator on the delegation log. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site and a copy will be placed in the patient's notes.

There will be one PIS and consent form for the study.

8.3. Registration

Following written informed consent, sites will allocate a unique trial number from a sequential list and complete a registration form. Once completed, this form will be emailed to MTw-tr.sentinustrial@nhs.net as soon as possible to register the participants with the trial office. Registration queries during normal working hours (Mon-Fri 9am to 5pm) can be dealt with by phoning 01622 939736.

Registration Service
Telephone: 01622 939736 (Mon–Fri 9am-5pm)
Mtw-tr.sentinustrial@nhs.net-

8.4. Baseline Assessments

Visit 0

Following written informed consent and registration, a contrast enhanced ultrasound and core biopsy +/- FNA of axillary sentinel lymph nodes (with clip marking) will be undertaken.

The patients will receive a standard after care leaflet about axillary nodal examination and biopsy as well as a satisfaction questionnaire to complete about the procedure.

Patients will be sent a questionnaire to fill in and post back (1 to 2 weeks after the procedure) to rate armpit pain and sensory disturbance in the armpit/ arm on a scale of 1-5. They will also be asked whether a bleeding or infective complication has occurred.

Visit 1

Results of the CEUS sentinel node biopsy.

Patients will be told the results of the CEUS sentinel lymph node biopsy. Based on this result and discussions at the MDT meeting, the patient and their direct clinical team will then plan their axillary surgery (see below).

Decisions regarding Axillary Management:

The results of the CEUS sentinel lymph node core biopsy will be discussed in the local MDT meeting and appropriate axillary surgical treatment decided with the following advice based on NICE guidance (1) and published evidence (18, 20):

- **Benign (B2) or inadequate (B1) SLN core biopsy result –** Proceed with surgical sentinel lymph node excision biopsy.
- **ITC only in SLN core biopsy specimen** – Proceed with surgical sentinel lymph node excision biopsy.
- **Micrometastasis (<2mm) in core biopsy specimen and eligible for axillary conservation –** Proceed with surgical sentinel lymph node excision biopsy. Consider localising clipped biopsied SLN prior to surgery to ensure metastatic lymph node is excised and confirm with X-ray of specimen.
- **Micrometastasis (<2mm) in core biopsy specimen and not eligible for axillary conservation** - Either offer targeted surgical sentinel lymph node excision biopsy with localisation of clipped biopsied SLN and specimen X-ray/ visual confirmation of clip removal or ALND with X-ray of resected specimen to ensure metastatic lymph node is excised. Discuss with patient balance of potential over-treatment with ALND (around 17% will have no further disease in the axilla) vs need for completion ALND if macrometastases found in resected SLN.
- **Macrometastasis (>2mm) in core biopsy specimen and eligible for axillary conservation** – Either offer targeted surgical sentinel lymph node excision biopsy with localisation of clipped biopsied SLN and specimen X-ray/ visual confirmation of clip removal or ALND with X-ray of resected specimen to ensure metastatic lymph node is excised. Discuss with patient balance of potential over-treatment with ALND (around 50% will have less than 2 macrometastases) vs need for completion ALND if macrometastases found in resected SLN. Consider X-ray of ALND specimen to confirm excision of malignant sentinel lymph node.
- **Macrometastasis (>2mm) in core biopsy specimen and not eligible for axillary conservation –** Proceed with ALND. Consider X-ray of specimen to confirm excision of malignant sentinel lymph node.

Visit 2 (surgery)

Breast surgery will be undertaken as standard of care with axillary surgery as directed above.

Patients will be sent a questionnaire to fill in and post back (1 to 2 weeks after the procedure) to rate armpit pain and sensory disturbance in the armpit/ arm on a scale of 1-5. They will also be asked whether a bleeding or infective complication has occurred.

Visit 3

Results of surgery

The results of surgery will be discussed in the local MDT meeting and further surgery/ adjuvant treatment will continue, as is the standard of care.

8.5. Discontinuation/Withdrawal of Participants from Trial intervention

Each participant has the right to withdraw from the trial at any time for any reason. Patients should be encouraged to remain within the trial, however if a patient wishes to withdraw, the Trial Office should be notified immediately. Any data acquired prior to withdrawal will be included in the final analysis (unless consent is withdrawn by the participant).

Withdrawal Criteria
Patients are free to stop study participation at any time without giving a reason. In the event of a participant's decision to withdraw from the trial, the Investigator should ascertain from which aspects of the trial the participant wishes to withdraw and record the details on the CRF. For participants withdrawing from all aspects of the trial, the investigator should ascertain from the participant if they continue to consent to collecting routine information from hospital records, and/or data linkage with existing databases e.g NHS Digital and eDRIS, cancer registries and national public health bodies.
Participants may withdraw from the trial intervention only; this may be at the discretion of the investigator due to safety concerns. If a participant is only withdrawn from the intervention, they must be followed-up in accordance with the protocol.
Participants moving away from the region of the local site should not automatically be withdrawn from the trial. Should this occur, please contact the Trial Office with details of the relevant participant, and they will endeavor to assign the participant's follow-up to a hospital site close to their new location. The participant's new contact address for postage of follow-up questionnaires will also need to be updated with the Trials Office.

In addition, the Investigator may discontinue a participant from trial intervention at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- Withdrawal of Consent

The reason for trial intervention withdrawal will be recorded in the CRF. If the participant is withdrawn from trial due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised. Participants withdrawing from the trial intervention will continue to be followed-up in accordance with the protocol.

8.6. Definition of End of Trial

The end of trial is marked by the point when the final patient is 30 days beyond the last scheduled visit (surgery).

9. TRIAL TREATMENTS

SonoVue ultrasound contrast agent (BRACCO International, Amsterdam, The Netherlands)

Marketing authorization number: EU/1/01/177/002

Molecular formula: F_6S

Sulphur Hexafluoride is a contrast agent composed of an inorganic fluorinated inert gas comprised of six fluoride atoms bound to one sulphur atom, with potential diagnostic activity upon imaging.

Physical description: Sulphur hexafluoride appears as a colorless odorless gas. Noncombustible. Shipped as a liquefied gas under own vapor pressure.

Decomposition: Sulfuryl and thionyl fluorides are the major decomposition products of sulfur hexafluoride.

Sulphur hexafluoride is an unreactive substance. Sulphur hexafluoride is not attacked by water, acids, or bases, at room temperature. It is resistant to the action of carbon, copper or magnesium at red heat, and will not react with sodium below its boiling point. It reacts with sulphur vapour or hydrogen at 400 °C.

ATC code: VO8DA05.

Pharmaceutcal properties: Sulphur hexafluoride is an inert, innocuous gas, poorly soluble in aqueous solutions. There are literature reports of the use of the gas in the study of respiratory physiology and in pneumatic retinopexy. The addition of sodium chloride 9 mg/mL (0.9%) solution for injection to the lyophilised powder followed by vigorous shaking results in the production of the microbubbles of sulphur hexafluoride. The microbubbles have a mean diameter of about 2.5 µ m, with 90% having a diameter less than 6 µ m and 99% having a diameter less than 11 µ m. Each millilitre of SonoVue contains 8 µ L of the microbubbles. The intensity of the reflected signal is dependent on concentration of the microbubbles and frequency of the ultrasound beam .The interface between the sulphur hexafluoride bubble and the aqueous medium acts as a reflector of the ultrasound beam thus enhancing blood echogenicity and increasing contrast between the blood and the surrounding tissues.

SonoVue has been shown to be rapidly removed from the blood. The route of SF6 elimination was by means of the lungs in the expired air.

Formulation: Sonovue sulphur hexafluoride microbubbles 8 uL/mL. Powder and solvent for dispersion of

injection. 1 vial containing 25 mg of powder to be reconsitituted with 5mL sodium chloride 9 mg/mL (0.9%) solution for injection.

Effects in humans

General contraindications: Hypersensitivity to the active substance (hexafluoride gas) or to any of the excipients (macrogol 4000, distearoylphosphatidylcholine, dipalmitoylphosphatidylglycerol sodium, palmitic acid).

Intravenous use:

Patients with unstable cardiopulmonary status

ECG monitoring should be performed in high-risk patients as clinically indicated. It is recommended to keep the patient under close medical supervision during and for at least 30 minutes following the administration of SonoVue. Use extreme caution when considering the administration of Sonvue in patients with recent acute coronary syndrome or clinically unstable ischaemic cardiac disease, including: evolving or ongoing myocardial infarction, typical angina at rest within last 7 days, significant worsening of cardiac symptoms within last 7 days, recent coronary artery intervention or other factors suggesting clinical instability (for example, recent deterioration of ECG, laboratory or clinical findings), acute cardiac failure, Class III/IV cardiac failure, or severe rhythm disorders because in these patients allergy like and/or vasodilatory reactions may lead to life threatening conditions. SonoVue should only be administered to such patients after careful risk/benefit assessment and a closely monitoring of vital signs should be performed during and after administration. It should be emphasised that stress echocardiography not only can induce an ischaemic episode but also the stressors may induce predictable, dose-dependent effects on the cardiovascular system (e.g., increase in heart rate, blood pressure and ventricular ectopic activity for dobutamine, or decrease in blood pressure for adenosine and dipyridamole) as well as unpredictable, hypersensitivity reactions. Therefore, if SonoVue is to be used in conjunction with stress echocardiography patients must have a stable condition verified by absence of chest pain or ECG modification during the two preceding days.
Moreover, ECG and blood pressure monitoring should be performed during SonoVue-enhanced echocardiography with a pharmacological stress (e.g. with dobutamine).

Other concomitant diseases

Caution is advisable when administering the product to patients with: acute endocarditis, prosthetic valves, acute systemic inflammation and/or sepsis, hyperactive coagulation states and/or recent thromboembolism, and end-stage renal or hepatic disease, as the numbers of patients with those conditions who were exposed to SonoVue in the clinical trials were limited.

Technical recommendation

In animal studies, the application of echo-contrast agents revealed biological adverse reactions (e.g. endothelial cell injury, capillary rupture) by interaction with the ultrasound beam. Although these biological side effects have not been reported in humans, the use of a low mechanical index is recommended.

Pregnancy
No clinical data on exposed pregnancies are available. Animal studies do not indicate harmful effects with respect to pregnancy, embryonal/foetal development, parturition or postnatal development. As a precautionary measure, it is preferable to avoid the use of SonoVue during pregnancy.

Breastfeeding
It is not known if sulphur hexafluoride is excreted in human milk. However, based on its rapid elimination from the body via the expired air, it is considered that the breastfeeding can be resumed two to three hours after administration of
SonoVue.

Fertility
No clinical data are available. Animal studies do not indicate harmful effects on fertility.

Supply of SonoVue

SonoVue will be purchased directly from the manufacturer by each site. The sponsor will provide and distribute labels to the study sites. The pharmacy at each site will label the immediate and outer packaging of the SonoVue before it is received in the breast ultrasound department for the purpose of the study.

Labelling
The following particulars will appear on the immediate and the outer packaging:

(a) Name, address and telephone number of the main contact for information on the product and clinical trial.
(b) The name of the substance and its strength or potency.
(c) Pharmaceutical form, route of administration, quantity of dosage units;
(d) The batch or code number identifying the contents and packaging operation;
(e) The subject identification number.
(g) Directions for use.
(h) 'For clinical trial use only'.
(j) Storage information.
(k) Period of use.

Handling of SonoVue for intradermal administration (trial procedure)

SonoVue has a shelf life of 2 years and does not require any special storage conditions.

SonoVue will be reconstituted as per the instructions stipulated in the summary of product characteristics (SmPC) with no alterations.

Once reconstituted, the chemical and physical stability has been demonstrated for 6 hours. The product should be used immediately after reconstitution to avoid microbial contamination. The vial is for single use only.

Do not use if the liquid obtained is clear and/ or solid parts of the lyophilisate are seen in the suspension.

Disposal

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

Administration (trial procedure)

Aseptic technique. 1% lignocaine is injected subcutaneously into the sub areola region. The contrast agent - “sonovue” is reconstituted as per the SmPC and up to 1ml is injected intra-dermally at the site of the local anaesthetic. The breast is gently massaged to encourage the contrast to be taken up by the lymphatics. The axilla is scanned and the contrast software package used on the ultrasound machine allows visualisation of the contrast agent into the axilla. The first draining lymph node is highlighted and biopsied using a 14G conventional core biopsy needle +/- fine needle aspiration. A marker clip may be placed into the lymph node to identify which node has been biopsied.

10. STATISTICS

We have assumed 25 cases per imaging specialist will be sufficient to skill up the workforce in terms of competency with the technique based on previous training packages. In order to progress to a National training scheme and future phase III trial, it is anticipated that at least 75% of imaging specialists will need to meet the following pre-specified standards; >85% visualization of tumour draining axillary SLN, >80% successful core biopsy (LN tissue retrieved) rate and >80% concordance with SLN identified surgically with SLNE.

A sample size of 235 is required to achieve a sensitivity of at least 56% with a prevalence of 22%, a 5% one sided significance level and 80% power, assuming a minimum sensitivity of 39% (REF: Cox Br J Radiology 2017).

An independent data manager will supervise centralized, prospective data collection. Anonymised descriptive statistics for the technical outcomes, using percentages and associated 95% confidence intervals, will be reported for each imaging specialist and overall. The total volume of axillary disease at the end of surgical treatment of those patients with a benign CEUS sentinel node core biopsy will be compared with those with an initial malignant CEUS sentinel core biopsy using Chi-squared tests or Mann-Whitney tests as appropriate. Individual unit and pooled detection rates of malignant lymph nodes using conventional B-mode axillary ultrasound will also be calculated. Diagnostic performance will be assessed by calculating the overall pooled sensitivity, specificity, positive predictive value, negative predictive value of CEUS SLN core biopsy as a test to identify SLN metastases as compared to axillary surgery and the prevalence of LN metastases.

11. DATA MANAGEMENT

11.1. Source Data

Source documents are where data is first recorded, and from which participants' CRF data are obtained. These will include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, electronic patient record and correspondence. Source data verification will be monitored to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki 1964 as amended October 1996. Monitoring by the Chief Investigator or authorised authorities will be to ensure

- Sufficient data is recorded to enable accurate linkage between hospital records and CRFs.
- Source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit.
- Staff working on the trial will meet requirements of the EU Directive.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions in accordance with the principle of GCP. On all trial-specific documents, other than the signed consent form, the participant will be referred to by the trial participant number and not by name.

11.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11.3. Data Recording and Record Keeping

Data Collection

Each site will be provided with an Investigator File containing Case Report Forms (CRFs). Data collected on each patient must be recorded by the local principal Investigator, or his/her designee, as accurately and completely as possible. The principal Investigator is responsible for the timing, completeness, legibility, accuracy and signing of the CRF and they will retain a copy of each completed form. The Investigators must allow study staff access to any required background data from hospital records (source data e.g. medical records) on request.

All fields MUST be completed. If a test or measurement was not done, please indicate why that was omitted on the CRF. Entries must be made in **black ballpoint pen**. Errors must be **crossed out with a single line** leaving the original data un-obscured (i.e. without overwriting), the correction inserted and the change initialled and dated. An explanatory note should be added if necessary. Correction fluid/tape/labels must not be used. All data submitted on CRFs must be verifiable in the source

documentation. These may include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, radiographs and correspondence. All documents will be stored in confidential conditions. Any deviation from this must be explained appropriately.

The imaging specialist participants and patients will be identified by unique trial specific numbers and/or codes in any database. The name and any other identifying detail will NOT be included in any trial data electronic file.

Completed CRFs should be returned to the Trial Office.

11.4. Data quality monitoring and audit

On receipt, all forms will be checked for completeness and congruity. Forms containing empty data fields or data anomalies will be queried with the site for resolution. Data will be entered onto the trial database and any further anomalies will be identified and queried with the site. Periodically, data will undergo additional checks to ensure consistency between data submitted on CRFs.

Trial staff will maintain regular communication with sites, through routine calls, mailings and/or meetings. In the event of persistent issues with the quality and/or quality of data submitted, an on-site monitoring visit may be arranged. In such circumstances, patient notes and the investigator site file must be available during the visit. The representative from the Trial Office will work with the site staff to resolve issues, offer appropriate training if necessary, and to determine the site's future participation in the trial.

An audit may be arranged at a site if the Trial Management Group feels it is appropriate. An independent team, determined by the Trial Management Group, will conduct audits.

11.5. Data storage

The local investigator must maintain documents not for submission to the trials unit (e.g. patients' written consent forms) in strict confidence. In the case of special problems and/or regulatory queries, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The trial office will maintain a trial database. This will contain all information related to trial participants including patient identifiable data. The database will meet industry-standard security criteria and will only be accessible to authorised personnel.

11.6. Data Sharing

The Trial Management Group supports the sharing of data with other researchers wishing to undertake additional analyses and will consider all formal requests for sharing data within this research. Once agreed, a data sharing agreement will be established between the Sponsor and recipient describing the conditions for data release and requirements for transfer, storage and publication to ensure that relevant intellectual property and the identity of individual trial participants are protected.

11.7. Archiving

All essential documentation and trial records will be stored by trial office in conformance with the applicable regulatory requirements and access to stored information will be restricted to authorised personnel. Trial documentation and data will be archived for at least 10 years after completion of the trial.

12. ADVERSE EVENT MANAGEMENT

12.1. Definitions

An Adverse Event (AE) is defined as any untoward medical occurrence in a trial participant and which does not necessarily have a causal relationship with their involvement in the trial.

A Serious Adverse Event (SAE) is an AE that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical condition.

An adverse reaction (AR) is an unwanted or harmful reaction, which occurs after administration of a drug and is suspected or known to be due to the investigational medical product (IMP).

A serious adverse reaction (SAR) is an AR that fulfils one of the following criteria and implies a reasonable possibility of a causal relationship between the event and the IMP.

- Results in death
- Is immediately life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical condition.

A suspected SAR refers to individual SAE case reports from clinical trials where a causal relationship between the SAE and the IMP was suspected by either the sponsor or investigator. All individually reported SARs are considered suspected. A SUSAR is a suspected unexpected serious adverse reaction.

12.2. Reporting

All adverse events must be graded according to the NCI Common Terminology Criteria for adverse events (NCI-CTCAE) Version 4.0.

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

Abnormal laboratory test results that are deemed clinically significant by the Investigator and that lead to a change in the trial intervention or temporary or permanent discontinuation of trial intervention, or require intervention or diagnostic evaluation to assess the risk to the subject should be recorded as adverse events and instigate further investigation and follow up as appropriate. An exacerbation of a pre-existing condition is an adverse event.

All adverse events must be followed until resolution or for at least 30 days after discontinuation of trial intervention (whichever comes first), or until toxicity has resolved to baseline or < Grade 1, or until the toxicity is considered to be irreversible. Perceived lack of efficacy is not an adverse event.

All SAEs that occur between trial entry and 30 days after surgery will be reported. If an unreported event from this time period is identified at a later date, retrospective reporting must occur immediately. Events occurring outside of this time period may still be reported if the Investigator feels that it is medically important.

SAEs will be reported using the SAE Form. The local Principal Investigator must report any SAEs to the Trial Office within 24 hours of becoming aware of the event. Do not delay reporting in order to identify causality or expectedness, which can be identified at a later stage and the report updated.

The SAE Form must be completed and faxed to the Trials office on: ***

In the absence of a responsible Investigator (as named on the Site Signature and Delegation Log), the SAE Form must be completed and signed by a member of the site trial team. The SAE Form must be checked by the responsible Investigator, signed and re-faxed as soon as possible.

The patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until the patient's status is unlikely to change further. Trial staff will liaise with the site to compile all of the necessary information and to resolve queries as necessary.

The Trial Office is responsible for reporting relevant events to the Sponsor, ethics committee and MHRA within required timelines in accordance with trial procedures and regulatory requirements. The PI is responsible for reporting events to local parties (e.g. R&D Department), in accordance with local practice.

All reportable events - serious and unexpected and drug related/relationship unknown, and any others as advised by the main REC, will be sent to Investigators for submission to relevant parties in accordance with local practice.

Trial staff will send a safety report to the main REC, MHRA and to the Sponsor annually. Sites should forward this report to their local R&D department in accordance with local practice and regulatory requirements.

If the event leads to the patient being withdrawn from trial medication, the appropriate CRF(s) must be completed in accordance with the CRF schedule. All SAEs will be subjected to a clinical review by the Chief Investigator (CI) and a clinical coordinator from WCTU to determine whether sufficient information has been provided and whether any further information should be requested. The Chief Investigator will review all adverse reactions for increased severity/frequency on a quarterly basis. Adverse event data will also be reviewed periodically by the Independent Data and Safety Monitoring Committee (IDSMC).

The following events do not require to be reported as SAEs:

- Hospitalisation or death due to cancer progression
- Hospitalisation for planned investigations
- Hospitalisation for drug administration or elective surgery

12.3. Death/Life-Threatening Events

In the case of death or life-threatening events, on the day of becoming aware of the event, please telephone or fax the Trial Office. The appropriate CRFs must be submitted in accordance with the CRF schedule.

In the case of death, where possible, a copy of the death certificate and post-mortem report (if applicable) should be submitted to the Trial Office as soon as possible. Names and hospital numbers must not be visible on these documents. The patient's trial number and initials must be clearly added to the document using black ball-point ink.

12.4. Investigator Assessment

Seriousness

When an AE/AR occurs, the responsible investigator must assess whether the event is classified as serious (i.e. an SAE).

Reference Safety Information

An expected event is defined as a known toxicity as listed in the SonoVue summary of product characteristics (SmPC) section 4.8. at the same severity/frequency.

Causality

The Investigator must assess the causality of all SAEs/SARs in relation to the trial intervention using the definitions below. The Sponsor will not be permitted to downgrade investigators' causality assessments (e.g. to change an investigator's assessment of an event from 'possible relationship' to 'unlikely to be related'). Events categorised as 'possible relationship', 'probable relationship' or 'definitely related' will be recorded and processed as 'related events'.

Relationship to study medication	Description
Unrelated	There is no evidence of any causal relationship
Unlikely to be related	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication or device). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment)
Possible relationship	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication or device). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments)
Probable relationship	There is evidence to suggest a causal relationship and the influence of other factors is unlikely
Definitely related	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out

12.5. Procedures in case of pregnancy

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the trial medication may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies and pregnancies of the partners of those patients recruited into the trial (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the trial. A Pregnancy Notification Form should be completed and submitted to the Trial Office. Follow-up information may be requested as necessary.

All reports of congenital abnormalities/birth defects must be reported and followed up as per the procedures for an SAE.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Sponsor

The sponsor is Maidstone and Tunbridge Wells NHS Trust.

13.2. Approvals

This study is a multicentre trial.

The protocol, informed consent form and participant information sheet will be submitted to an appropriate Research Ethics Committee (REC) and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

13.3. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the REC, host organisation and Sponsor.

13.4. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained and only the minimal identifiable information will be collected. The imaging specialist participants and patients will be identified only by initials and a participants ID number on the CRF and any electronic database. Imaging specialists can request their own performance data. Participant's date of birth will be collected to calculate age. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

13.5. SERIOUS BREACHES

A serious breach is defined as "A breach of the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee and the NHS host organisation within seven calendar days.

13.6. Expenses and Benefits

Patients will be offered a £30 contribution towards their travel expenses.

14. FINANCE AND INSURANCE

14.1. Funding

The trial is funded by Breast Cancer Now.

14.2. Insurance

NHS indemnity through the Clinical Negligence Scheme for Trusts (CNST).

15. Trial Organisation

15.1. Trial Management Group (TMG)

The TMG includes the co-investigators, who are a multidisciplinary team of clinicians, statisticians and a patient advocate with considerable expertise in all aspects of design, running, quality assurance and analysis of the trial. It is anticipated that the TMG will meet monthly by teleconference.

15.2. Trial Steering Committee (TSC)

The TSC will have an independent Chairperson. TSC meetings will be held at regular intervals determined by need but not less than once a year. Routine business is conducted by email, post or teleconferencing. Members of the TMG will be co-opted onto the TSC as appropriate.

The Trial Steering Committee will take responsibility for:

- Major decisions such as a need to change the protocol for any reason
- Monitoring and supervising the progress of the trial
- Reviewing relevant information from other sources
- Informing and advising on all aspects of the trial.

15.3. Patient and Public Involvement (PPI)

PPI involvement is fundamental to design and in the development of all patient focussed information including patient information sheets and the dissemination of the results of the study. Sophie Gasson, as a Co-investigator and a member of the Independent Cancer Patients Voice (ICPV), has been involved in discussion regarding the study design to ensure that the study is acceptable to patients and is a member of the TMG.

16. Dissemination and Publication

The results of the trial will be published in peer-reviewed journals and presented at National and international meetings. The main report will be drafted by the Trial Management Group, and the final version will be agreed by the Sponsor/Funder before submission for publication, on behalf of the collaboration.

The success of the trial depends on the collaboration of doctors, nurses and researchers from across the UK. Equal credit will be given to those who have wholeheartedly collaborated in the trial.

The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).

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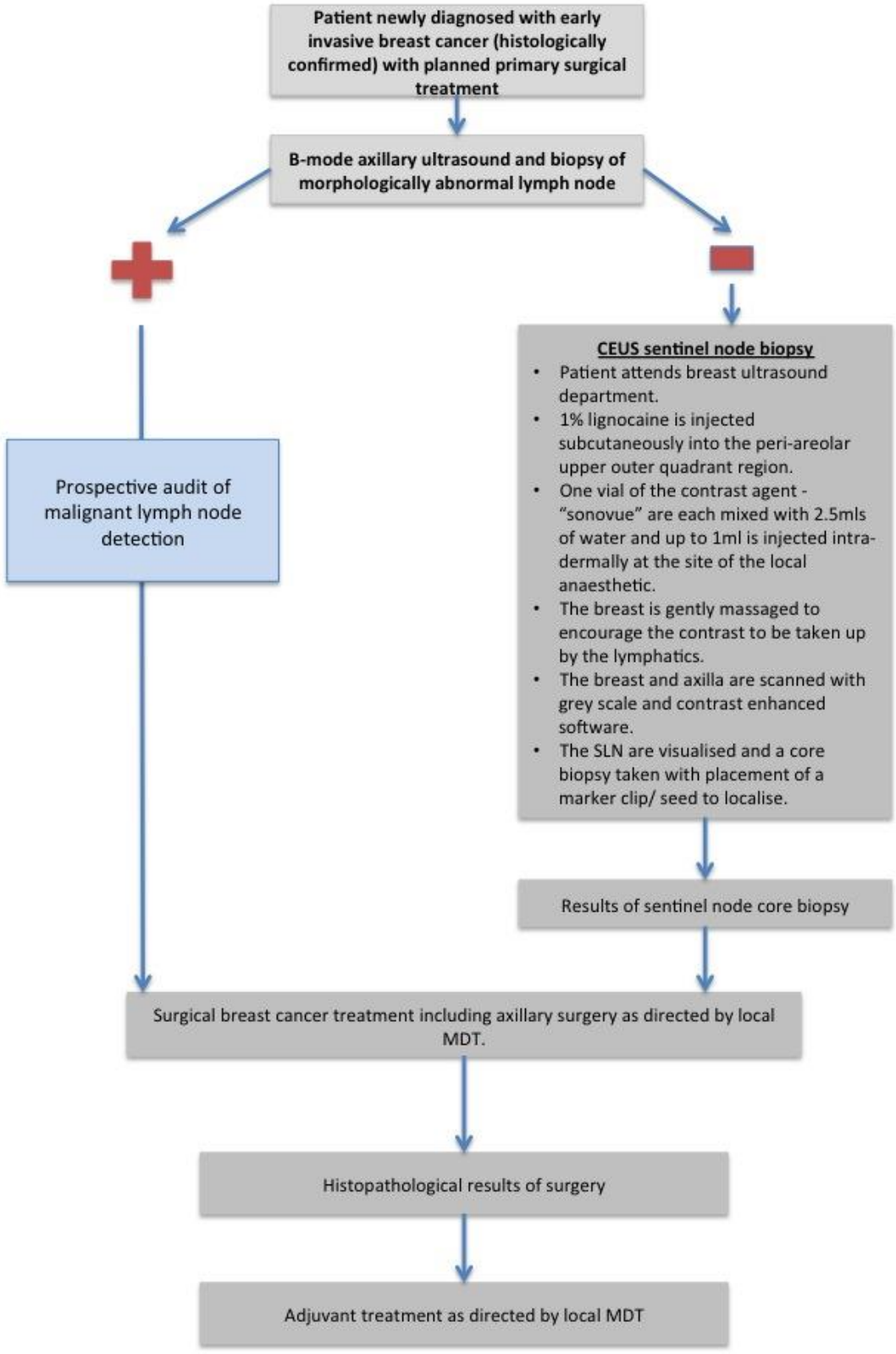
18. APPENDIX A: FLOW CHART SHOWING COMPARISON OF TRIAL PATHWAY VERSUS STANDARD OF CARE.



18. APPENDIX B: SCHEDULE OF PROCEDURES

Procedures	**Visits**				
	Screening	**Baseline**	**Visit 1**	**Visit 2**	**Visit 3**
Informed consent	X				
Eligibility assessment	X				
CEUS and sentinel node biopsy		X			
Satisfaction Questionnaire		X			
Post-procedure Questionnaire		X			
Results of CEUS sentinel node biopsy			X		
Surgery				X	
Post-armpit surgery questionnaire				X	
Results of surgery					X

19. APPENDIX D: AMENDMENT HISTORY

Amendment No.	**Protocol Version No.**	**Date issued**	**Author(s) of changes**	**Details of Changes made**
1	5.0	15th December 2020	Karina Cox	Participating sites to purchase IMP (SonoVue) rather than sponsor purchase and distribute IMP. Bristol to be removed as a site. PI at Barts changed from Jennifer Hu to Tamara Suaris. PI at MTW to be changed from Karina Cox to Nicky Dineen.
2	5.1	24th May 2021	Karina Cox	Addition of Logo and formatting changes to protocol and patient facing documents University Hospitals Coventry and Warwickshire NHS Trust (UHCW) to be added as a site and Dr Muthyala Sreenivas added as the PI for UHCW. PI at Guys' Hospital NHS Trust changed from Dr Ali Sever to Mr

				Ashutosh Kothari.
3	5.2	1st December 2021	Karina Cox	Addition of St James's Leeds and Guy's Hospital London as sites to train radiologists to perform the test procedure. Participating radiologists can perform a fine needle aspiration biopsy as well as a core biopsy of sentinel lymph nodes at their discretion. A study website has been developed and will contain information for patients as well as the radiology training videos. PI at Maidstone and Tunbridge Wells NHS Trust changed from Dr Nicky Dineen to Dr Jenny Weeks. Registration information changed to make email registration the primary method to register the patient.
4	5.3	27th July 2023	Karina Cox	5 sites are either open or in the later stages of set-up but because of ongoing pressure on clinical and research teams, recruitment at some sites is slow. To mitigate this, the trial management group have decided to allow high recruiting sites to involve more than 2 imaging specialists. As long as each specialist performs 25 procedures the statistical integrity of the study remains intact. This also allows for the possibility that one of the 5 sites will not manage to recruit at all. Because of the pressure on clinical and research teams, the trial management group have decided to drop the requirement for participating sites to audit their malignant lymph node detection rate with conventional B-mode ultrasound and biopsy. Although the information would have been interesting, this audit is not needed for the final statistical analysis and the lack of this information will not affect the integrity of the study. Instead, the trial management group feel that auditing the time taken from breast cancer diagnosis to a patient knowing whether their armpit lymph nodes contain cancer

				would provide useful information for the SENTINUS study and help with ongoing PPI work. Anecdotally, patients say that waiting for results is very hard and patients enrolled in the SENTINUS study are likely to know their armpit lymph node status many weeks before those on a standard diagnostic pathway. The section on 'Decisions regarding axillary management' has been updated to reflect current surgical practice and concerns about over-treatment of the axilla. In addition, the updated section is laid out more clearly to help clinical teams make decisions about the management of armpit lymph nodes in patients with early breast cancer. For most patients, the test procedure will involve an extra visit to the hospital. Therefore, patient representatives felt that a contribution towards travel expenses (£30) would help with recruitment. Because of the time pressures in clinic, surgical teams are finding it difficult to fill out the post procedure/ armpit surgery questionnaires in clinic. Following a sponsor monitoring meeting, it was deemed more effective if questionnaires (with a covering letter) could be sent to patients to fill out at home. They could then send them back to the research team. The patients would not need to pay for this. The PI at Maidstone and Tunbridge Wells NHS Trust has been changed from Dr Jenny Weeks to Karina Cox. The funder has granted a no cost extension for the study until the 5th of January 2024.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee or MHRA.