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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Ethics Ref:

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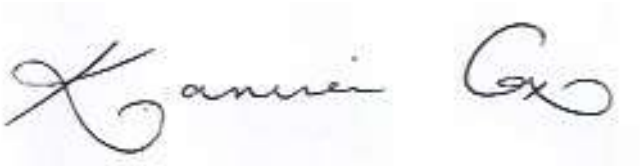

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1. KEY TRIAL CONTACTS

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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	50 breast cancer patients from 5 Breast Units (250 in total).	
Participating imaging specialists	10 from 5 breast units (2 per unit).	
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 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 mixed with 2.5mls of water 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. 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
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
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 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 to 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 5 Breast Cancer Units within the UK. Two 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 3 units will be naïve to the technique. Each unit will put forward 2 imaging specialists to take part in the study, therefore 10 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 an all-day training session at Maidstone Hospital, Kent. They will have access to video tutorials and written information. Dr Jenny Weeks and Dr Nisha Sharma will provide mentorship either by telephone or site visits if necessary.

During the trial period, each unit will also prospectively audit their malignant lymph node detection rate with conventional B-mode axillary ultrasound and biopsy.

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 50 patients over 24 months (250 in total) with each of the 10 participating imaging specialists performing 25 procedures.

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 complete a registration and eligibility form and register the participants with the trial office. During registration, eligibility will be checked and a unique trial number allocated.

Registration Service
Telephone: (Mon-Fri 9am-5pm)
Fax:

8.4. Baseline Assessments

Visit 0

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

Research study: Aseptic technique. 1% lignocaine is injected subcutaneously into the sub areola region. The contrast agent - "sonovue" is mixed with 2.5mls of water and 1mls 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. A marker clip is placed into the lymph node to identify which node has been biopsied.

The patients will receive a standard after care leaflet about axillary nodal examination and biopsy.

Visit 1

Results of the CEUS sentinel node core biopsy.

Axillary management: The result of the CEUS sentinel 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): If the sentinel node core biopsy sample is benign or contains isolated tumour cells/ micrometastases then sentinel lymph node excision is recommended; if a macrometastasis is found in the sentinel node core biopsy sample but the patient is eligible for axillary conservation then sentinel lymph node excision is recommended +/- completion axillary lymph node dissection or axillary radiotherapy as directed by the local MDT; if a macrometastasis is found in the sentinel node core biopsy sample but the patient is not eligible for axillary conservation then a primary axillary lymph node dissection is recommended.

Visit 2 (surgery)

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

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. Full details of the reasons for withdrawal must be recorded on the relevant CRF. Any data acquired prior to withdrawal will be included in the final analysis (unless consent is withdrawn by the participant).

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. 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.

10. DATA MANAGEMENT

10.1. 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.

10.2. 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.

10.3. 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.

10.4. 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.

10.5. 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.

10.6. 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.

10.7. 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.

11. ADVERSE EVENT MANAGEMENT

11.1. 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.

11.2. 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

11.3. 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.

11.4. Investigator Assessment

Seriousness

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

Expectedness

An expected event is defined as a known toxicity as listed in the Investigator Brochure/Summary of Product Characteristics 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

11.5. 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.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Sponsor

The sponsor is Maidstone and Tunbridge Wells NHS Trust.

12.2. 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.

12.3. 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.

12.4. 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.

12.5. 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.

12.6. Expenses and Benefits

No expenses are provided for the study.

13. FINANCE AND INSURANCE

13.1. Funding

The trial is funded by Breast Cancer Now.

13.2. Insurance

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

14. Trial Organisation

14.1. 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.

14.2. 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.

14.3. 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.

15. 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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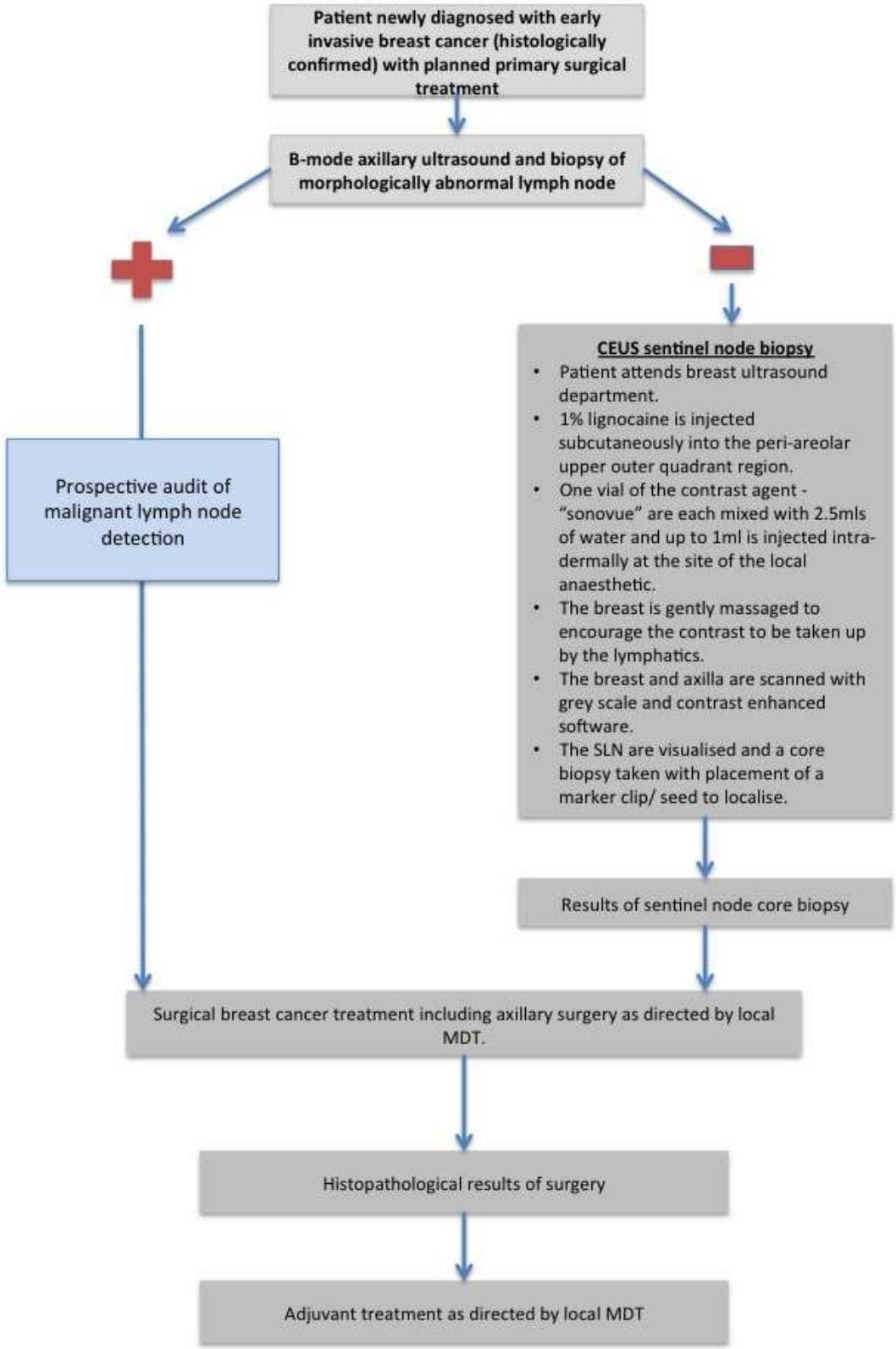
18. APPENDIX A: FLOW CHART SHOWING COMPARISON OF TRIAL PATHWAY VERSUS STANDARD OF CARE.



17. 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			
Results of CEUS sentinel node biopsy			x		
Surgery				x	

18. APPENDIX D: AMENDMENT HISTORY

Amendment No.	**Protocol Version No.**	**Date issued**	**Author(s) of changes**	**Details of Changes made**

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