FULL/LONG TITLE OF THE TRIAL

A prospective randomised study comparing indocyanine green (ICG) fluorescence combined with a standard tracer versus ICG alone for sentinel lymph node (SLN) detection in early breast cancer

SHORT TRIAL TITLE / ACRONYM

Indocyanine green Node FLUorEsceNCE study: INFLUENCE

INFLUENCE PROTOCOL V1 06-10-21

This protocol has regard for the HRA guidance and order of content V1.2 March 2016

RESEARCH REFERENCE NUMBERS

IRAS Number:	301478
ISRCTN Number:	
SPONSORS Number:	3.014.21
FUNDERS Number:	

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Good Clinical Practice (GCP) guidelines, the Sponsor's (and any other relevant) Standard Operating Procedures, and other regulatory requirements.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Trial Sponsor

Signature:

.....

Date:
...../...../.....

Name: Patricia Burns

Position: Senior Research Governance Manager

Chief Investigator

Signature: V. Pitsinis

.....

Date:
7/10/2021

Name: Mr Vassilis Pitsinis:

Statistician

Date:
.07/10/2021.....

Signature:

Name: Dr Petra Rauchhaus

Position: Clinical Trials Statistician

KEY TRIAL CONTACTS

Chief Investigator	Mr Vassilis Pitsinis Breast Unit Surgical Offices, Level 6, Ninewells Hospital and Medical School, Dundee DD1 9SY 01832 383876 Vasileios.Pitsinis@nhs.scot
Trial Co-Ordinator	Margaret Band Senior Trial Manager, Tayside Clinical Trials Unit (TCTU) 01382 383297 m.band@dundee.ac.uk
Statistician	Dr Petra Rauchhaus, Tayside Clinical Trials Unit P.Rauchhaus@dundee.ac.uk
Sponsor	Patricia Burns Research & Development Office, Tayside Medical Science Centre (TASC), Residency Block Level 3, George Pirie Way, Ninewells Hospital, Dundee DD1 9SY, 01382 383297, TASCgovernance@dundee.ac.uk
Funder(s)	Association of Breast Surgery The NHS Tayside Breast Unit Endowment Fund
Clinical Trials Unit	Tayside Clinical Trials Unit, TASC, Residency Block, Level 3, Ninewells Hospital, Dundee, DD1 9SY 01382 383581, TCTU@dundee.ac.uk
Key Protocol Contributors	Mr Vassilis Pitsinis - CI Prof John Benson - Collaborator Dr Fiona Hogarth – Co-Director, TCTU Dr Petra Rauchhaus – Statistician Margaret Band – Senior Trial Manager

LIST of CONTENTS

I. LIST OF ABBREVIATIONS

AR Adverse Reaction

CI Chief Investigator

CNORIS Clinical Negligence and Other Risks Insurance Scheme

CRF Case Report Form

DMS Data Management System

GCP Good Clinical Practice

ICF Informed Consent Form

ICG Indocyanine Green

ISF Investigator Site File

ISRCTN International Standard Randomised Controlled Trials

MedDRA Medical Dictionary for Regulatory Authorities

NHS R&D National Health Service Research & Development

PDE Photo Dynamic Eye

PI Principal Investigator

PIS Participant Information Sheet

REC Research Ethics Committee

SLN Sentinel Lymph Node

TASC Tayside medical Science Centre

TCTU Tayside Clinical Trials Unit

TMF Trial Master File

TMG Trial Management Group

TSC Trial Steering Committee

II. TRIAL SUMMARY

Trial Title	**A prospective randomised study comparing indocyanine green (ICG) fluorescence combined with a standard tracer versus ICG alone for sentinel lymph node (SLN) detection in early breast cancer**	
Internal ref. no. (or short title)	Indocyanine green Node FLUorEsceNCE study: INFLUENCE	
Trial Design	Multicentre, 2-arm, randomised, controlled intervention versus standard care	
Trial Participants	Women diagnosed with invasive breast cancer	
Planned Sample Size	100	
Treatment duration	Single intra-operative intervention	
Follow up duration	Routine post-operative review at 2 weeks and telephone consultation at 3 months	
Planned Trial Period	3 months	
	Objectives	Outcome Measures
Primary	To determine the sensitivity of fluorescence imaging alone for SLN identification compared with a combination of ICG and a standard tracer (blue dye or radioisotope)	Percentage of patients with successful identification of the SLN using ICG alone or combined with a standard tracer, stratified by cohort
Secondary	Procedural node positivity rates	Proportion of SLN biopsy cases with tumour deposits in at least one node (including macrometastases, micrometastases and isolated tumour cells)
	Adverse events from SLN biopsy	Seroma formation Cutaneous staining
Intervention	ICG fluorescence as single tracer	

III. FUNDING AND SUPPORT IN KIND

FUNDER(S)

Association of Breast Surgery

The NHS Tayside Breast Endowment Fund

IV. ROLE OF TRIAL SPONSOR AND FUNDER

The sponsor assumes overall responsibility for the initiation and management of the trial.

Trial design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results will remain the responsibility of the sponsor. The sponsor will maintain the right for the final decision regarding any of these aspects of the trial. Funding provided does not infer any governance of the research or supervision of the conduct of the research by the funders.

V. ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

The trial will be coordinated by a Trial Management Group (TMG), consisting of the grant holders, including the CI, collaborators, statistician, trial manager and research nurse where appropriate. Details of membership of the TMG will be held in the Trial Master File (TMF). The TMG will meet regularly to ensure all practical details of the trial are progressing well and working well and everyone within the trial understands them. Minutes of the TMG meetings will be maintained in the TMF.

The functions of the Trial Steering Committee (TSC) will be undertaken by the TMG. No independent TSC will be convened for this trial.

The functions of the Data Monitoring Committee will be undertaken by the TMG. No independent DMC will be convened for this trial.

The CI will be responsible for the conduct of the trial. Site delegate(s) will oversee the trial and will be accountable to the CI. A trial-specific Delegation Log will be prepared for each Site, detailing the duties of each member of staff working on the trial.

The trial will be conducted in accordance with the principles of GCP.

In addition to Sponsorship approval, a favourable ethical opinion will be obtained from an appropriate NHS REC. Authorisation from appropriate National Health Service Research & Development (NHS R&D) permission(s) will be obtained prior to commencement of the trial.

VI. PROTOCOL CONTRIBUTORS

Chief Investigator: Mr Vassilis Pitsinis, Initial draft, review and final approval

Co-investigator: Prof John Benson, Review

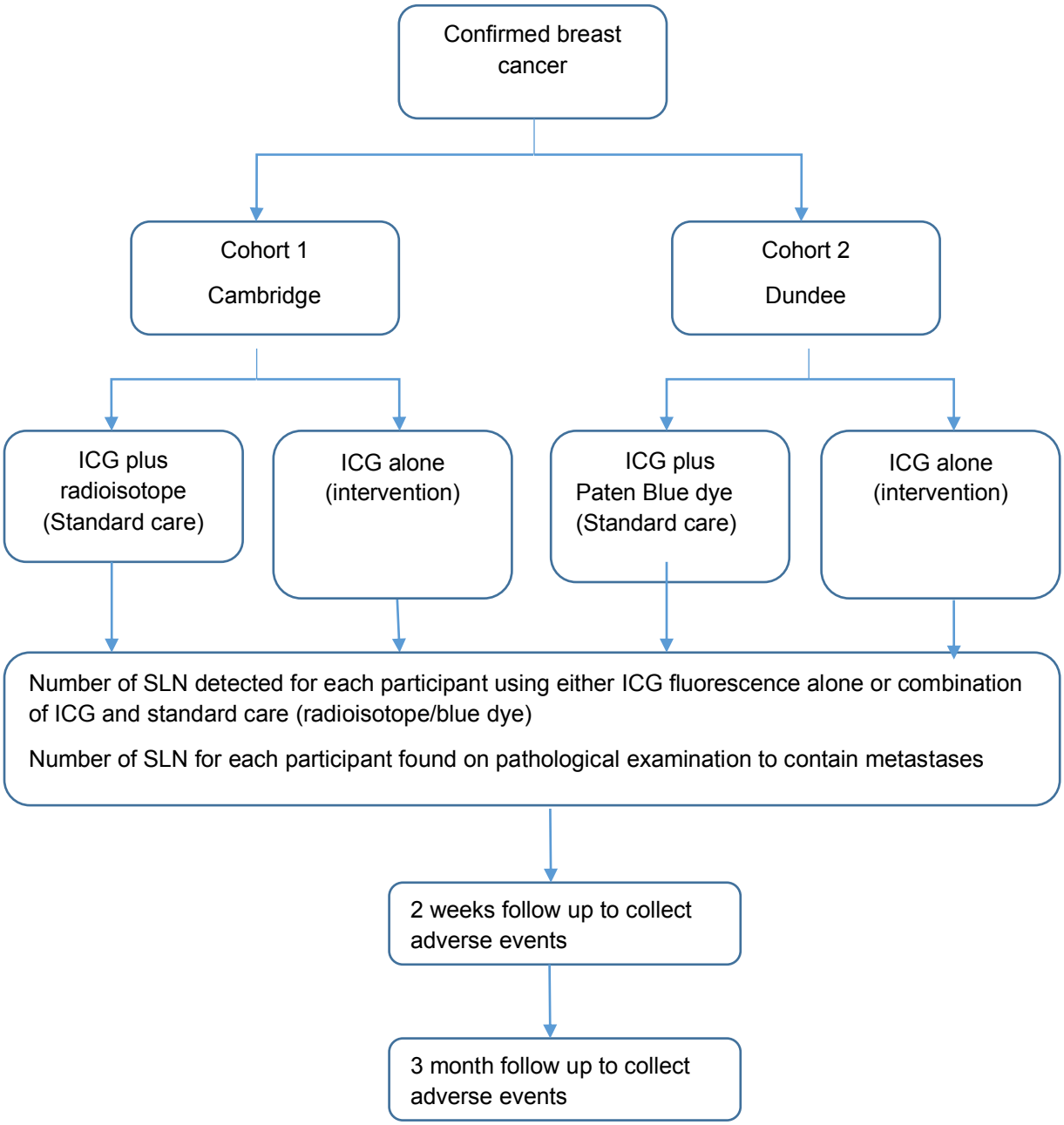
TCTU Co Director: Dr Fiona Hogarth, Review

TCTU Statistician: Petra Rauchhaus, Review

TCTU Senior Trial Manager: Margaret Band, Review

VII. KEY WORDS: fluorescence, sentinel lymph node (SLN), breast cancer

VIII. TRIAL FLOW CHART



1. BACKGROUND

Fluorescence navigation using the fluorochrome ICG has been employed to visualise lymphatic channels and nodal tissue for identification of SLN(s) in breast and other site cancers. The fluorescent signal is captured by a photodynamic eye composed of a series of light emitting diodes (LEDs) that produce light at a wavelength of 760nm, a lens and a filter. The detector is a charge coupled device camera that filters out wavelengths below 820nm. The fluorescent signals appear on a TV monitor. Fluorescence develops immediately following intra-dermal/subareolar injection of ICG and within seconds can be seen in the subcutaneous vessels of the breast. The illuminated subcutaneous lymphatic channels can be visualised on the Photo Dynamic Eye (PDE) display and progression of the ICG within the lymphatic system tracked as it passes towards the axilla. The fluorescence is scattered by superficial tissues and cannot be detected at a depth of more than 1cm with current technology. The fluorescent signal is lost from scattering and technical improvements are aimed at reducing scatter and improving sensitivity (e.g. development of a PDE which can detect fluorescence at a depth of up to 2cm). Pressure applied to the axillary region enables lymph nodes to be visualised percutaneously before the skin incision in made. The fluorescent lymphatic channels disappear around the level of the lower axilla and can otherwise only be detected (together with sentinel nodes) after the skin incision has been made. This point at which the subcutaneous lymphatic channels cease to be seen is used to determine the site of skin incision. Application of pressure may permit visualisation of a sentinel node through the skin, but otherwise an incision is made at the point where the fluorescent lymphatics disappear.

Early validation studies used ICG alone with reports of identification rates in excess of 90%. Subsequent practice has favoured a combination of ICG with either blue dye or radioisotope that allows both conventional and fluorescent visualisation of the lymphatic vessels and nodes. These have all shown high levels of nodal recognition by fluorescence with few nodes (<5%) being classified as blue and/or hot but not fluorescent. The ICG-10 study confirmed that combined nodal sensitivity is higher for blue dye and ICG (95.0%) compared with the standard combination of blue dye and radioisotope (73.1%).

Recent studies have shown high levels of concordance (>90%) for SLN detection not only between ICG and blue dye but also ICG and radioisotope. Fluorescence imaging provides at least equivalent detection rates but offers an additional dimension to the technique of SLN biopsy and is safe with in frequent allergic reactions. The combination of ICG with a standard tracer agent represents a transition phase and we now wish to explore ICG as a sole tracer having accrued clinical experience with its usage. In a comparison of ICG as a sole tracer versus radioisotope performed in Japan identification rates for ICG alone and radiocolloid were 98.7% and 96.7%respectively.ICG combines many of the advantages of blue dye and radioisotope without the disadvantages. Tracer combinations with dual localisation are more expensive and inconvenient than ICG alone but not necessarily associated with improved accuracy that is significant in terms of clinical outcomes.

2. RATIONALE

More than half of newly diagnosed breast cancer patients will undergo routine SLN biopsy annually in the UK (>25,000 cases). Blue dye is becoming less popular as a tracer due to potential allergic reactions and staining of cutaneous/breast tissues. Drawbacks of radioisotope include availability, cost, patient inconvenience/discomfort, radiation exposure/disposal and mandatory licensing. Fluorescence navigation has high optical sensitivity and permits a real-time sequential SLN dissection guided by visualisation of lymphatic tissue and sentinel nodes that is not possible with radioisotope alone. In particular, identifying sentinel nodes in order of ‘biological’ priority is more difficult with radioisotope whereby nodes are

detected as hotspots irrespective of anatomical lymphatic flow. The fluorescent node(s) acts as a 'beacon' that can aid dissection within a fatty axilla (patients with high body mass index). Hence there are potential drawbacks from use of both blue dye and radioisotopes for SLN localisation in breast cancer. Moreover, radioisotopes are a by-product of a contracting nuclear industry and supply might become unpredictable with widespread usage in the emerging economies of India, China and Brazil. This may lead to increased costs and render alternative tracer agents more attractive in terms of surgical technique, safety and patient convenience/comfort but also cost-effectiveness. The proposed study could be a forerunner to a larger multicentre trial evaluating ICG alone. Localisation techniques using a non-radioactive tracer with a real-time visual component warrant further investigation to confirm equivalence in performance compared to standard tracer agents.

ICG is a less expensive tracer than radioisotope although initial capital equipment costs (PDE camera) are higher. Nonetheless, in the longer-term use of ICG is likely to be more cost-effective than radioisotope taking account cost of the radioisotope injection per patient. Moreover, in a COVID-19 environment, additional visits to the nuclear medicine department can be avoided with use of ICG, whether this is on the day of surgery or before. The eventual use of ICG in place of radioisotope has the potential benefits of avoiding radioactive materials and problems of waste disposal and monitoring. Dispensing with the requirements for radioisotope facilities and mandatory licensing could save the NHS between £100 and £200 per patient. It is also important to stress that this is just for the cost of the radiocolloid injection. In addition to potential cost savings from avoiding necessity of a nuclear medicine facility, there are logistical advantages relating to booking and organising appointments for radiocolloid injection whether this be the day before or same day as surgery.

It is hypothesized that fluorescence mapping can provide at least equivalent sentinel detection rates but offer the opportunity for avoiding blue dye and eventually lead to improved cost-effectiveness if radioisotope is eventually abandoned for routine SLN biopsy. Abolition of blue dye with its associated risk of anaphylactic reaction (1-3%), skin tattooing and non-licensed use will be notable benefits of ICG. Of note, in a UK-Turkish study blue discoloration persisted at the injection site after 12, 24, and > 36 months in 36.5, 23.6, and 8.6% of the patients respectively.

2.1. Assessment and Management of Risk

Standard SLN detection techniques involve the use of blue dye or radioisotopes. ICG fluorescence detection is an alternative tracer agent that has been used in combination with these standard techniques. Participants in this study will be divided amongst two cohorts. Participants in Cohort 1 will be randomised to either ICG alone (intervention) or ICG plus radioisotope (standard care). Participants in Cohort 2 will be randomised to ICG alone (intervention) or ICG plus blue dye (standard care). There is no evidence that any participants randomised to intervention are at no higher risk than those on standard care.

This trial is categorised as:

Type A = No higher than the risk of standard medical care

See Appendix 1

3. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Hypothesis

ICG as a sole fluorescence tracer provides equivalent detection rates when compared to standard detection techniques using blue dye or radioisotopes in combination with ICG.

3.1. Primary objective

To determine the sensitivity of ICG fluorescence imaging alone for SLN detection compared to a combination of ICG and standard tracer.

3.2. Secondary objectives

To determine procedural and node specific positivity rates for intervention and standard care.

3.3. Outcome measures/endpoints

See section 3.6

3.4. Primary endpoint/outcome

See Section 3.6

3.5. Secondary endpoints/outcomes

See Section 3.6

3.6. Table of endpoints/outcomes

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective To determine the sensitivity of fluorescence imaging alone for SLN identification compared with a combination of ICG and a standard tracer (blue dye or radioisotope)	Percentage of patients with successful identification of the SLN using ICG alone or combined with a standard tracer, stratified by cohort	At the time of SLN biopsy
Secondary Objectives Procedural node positivity rates	Proportion of SLN biopsy cases with tumour deposits in at least one node (including macrometastases, micrometastases and isolated tumour cells)	Post-surgery, when histopathology results are available
Adverse events from SLN biopsy	Seroma formation Cutaneous staining Other adverse reactions to tracers	2 weeks and 3 months

4. TRIAL DESIGN

This is a multicentre (two site), randomised, controlled trial. Participants will be recruited within two cohorts, one cohort at each site. Each cohort consists of parallel groups, with two treatment arms, intervention versus standard care. A total of 100 participants will be randomised, 25 in each group.

The treatment arms for each cohort are as follows:

Cohort 1 (Cambridge)

1. ICG 2mls 0.5%

2. ICG 2mls 0.5% combined with radioisotope Technetium^{99} nanocolloid, 20 MBq

Cohort 2 (Dundee)

1. ICG 2mls 0.5%
2. ICG 2mls 0.5% combined with Patent Blue dye 2mls 2.5%

Patients will continue with standard care for further treatment of their condition.

5. TRIAL SETTING

This study will recruit patients from 2 large UK teaching hospitals with high volumes of breast cancer patients. Participants will be identified from the breast cancer service at these hospitals and may include at weekly multi-disciplinary team meetings attended by surgeons, radiologists, pathologists, breast care nurses and research staff.

6. PARTICIPANT ELIGIBILITY CRITERIA

6.1. Inclusion criteria

- Female
- Over 18 years of age
- Biopsy proven invasive breast cancer
- Tumour(s) measuring < 5cm in radiological size
- No record of clinical or sonographic evidence of abnormal axillary lymph nodes
- Planned SLN biopsy to be carried out as per local standard care using ICG plus radioactive tracer (Cambridge only) or ICG plus Patent Blue Dye (Tayside only)

6.2. Exclusion criteria

- Neoadjuvant chemotherapy
- Prior ipsilateral axillary surgery or breast excision biopsy
- Pregnant or breast feeding

Allergy to the tracers, pregnancy or breast feeding are not described as exclusion criteria as these patients will be excluded from SLN biopsy standard care.

7. TRIAL PROCEDURES

7.1. Recruitment

100 female adults, $\geq$ 18 years of age with biopsy proven invasive breast cancer will be randomised.

Sites will be informed when the target number of study participants has been reached and will be instructed not to recruit any further participants. All participants already consented to take part at this point will go forward to randomisation and will be followed until the end of the trial.

Sites will maintain a recruitment log to track potentially eligible patients who are approached about the trial but are not randomised. This data is required for the Consolidated Standards of Reporting Trials (CONSORT). Anonymised information on participants who are not randomised will include:

- Age
- Whether the patient consented

- The reason the patient was not eligible for trial participation or if they are eligible but declined, with reason for declining if provided

7.1.1. Participant identification

Identification of potentially eligible participants will be a member of the clinical team. The staff will identify patients within the breast cancer service at weekly breast MDT meetings or clinic appointments who have been diagnosed with invasive breast cancer and scheduled for routine SLN biopsy as part of primary surgical management.

7.1.2. Screening

No trial specific screening procedures are required other than to confirm participants meet the inclusion criteria.

7.1.3. Ineligible participants

Where an individual is found to be ineligible for trial participation, they will be thanked and reasons for ineligibility fully explained. Any queries or questions will be answered by an appropriate member of the research team. If ineligibility is related to an incidental finding which is clinically significant, it will be reported to the participant's GP and/or consultant by the CI or Site Principal Investigator (PI), with consent of the individual.

7.1.4. Payment

No payment will be provided to participants as all assessments will be carried out either whilst already in hospital as an inpatient/day case or by telephone consultation after discharge.

7.2. Consent

The PI retains overall responsibility for the conduct of research at their site and this includes taking of informed consent at each site. They must ensure that any person delegated responsibility for seeking informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of GCP and Declaration of Helsinki.

Participants will receive a copy of the Participant Information Sheet (PIS) and ICF and will be given at least 24 hours to consider participation in the trial prior to consent. Prior to consent participants will be given the opportunity to ask any questions about the trial. Where a participant requests to speak with a physician from the trial team the consent process will not be completed until the participant had spoken to the physician and had all questions answered to their satisfaction. Once a patient agrees to participate, written informed consent will be obtained and eligibility confirmed.

In Cambridge, participants will be phoned after they have received the PIS and the consent process will be completed over the phone. This is to allow the participant to be randomised and informed whether they will need to attend a clinical visit to receive the radioisotope or not. The consent process will be witnessed by a member of staff independent of the trial team. The PI (or delegate) will sign the consent form as the person receiving consent and the independent witness will sign the consent form to confirm that the consent process was followed and the participant gave their verbal consent to take part. Participants will be asked to sign the consent form when they come for their surgery. A copy of the consent form will be given to them at this point.

In Tayside, participants will complete the consent process in person when they attend for their biopsy surgery.

The original Informed Consent Form (ICF) will be filed in the TMF or Investigator Site File (ISF) and a copy will be given to the participant and a copy will be filed in the participant's medical notes.

Where a participant loses capacity to consent the participant will be withdrawn from the study. Identifiable data or tissue already collected with consent will be retained and used in the study. No further data or tissue will be collected or any other research procedures carried out on or in relation to the participant. Participants are free to withdraw from the trial at any time. The reasons, if known, will be recorded in the medical case notes and Case Report Form (CRF).

Although a participant is not obliged to give reason(s) for withdrawing prematurely, if the participant appears lost to follow up, the CI will make a reasonable effort to ascertain the reason(s), while fully respecting the individual's rights, and will demonstrate that everything possible was done in an attempt to find any participant lost to follow-up. Those lost to follow-up or withdrawn will be identified but will still be included in the analysis if they received the intervention.

7.2.1. Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

Not applicable

7.3. The randomisation scheme

Prior to commencement of the trial, a randomisation list will be generated for both sites. Randomisation allocation will be 50:50 intervention: standard care for each cohort. The allocation arm for each participant will be placed into sealed double envelopes with the participant specific identification number on the outside; each envelope will only contact the allocation for one participant. There will be no stratification or minimisation.

At the time of consent, participants will be allocated a unique participant identification number. After consent and prior to randomisation, the exclusion criteria will be checked. This will be documented in the CRF.

Participants in each Cohort will be randomised by the PI or delegate to one of two options as detailed in Section 8. The outcome of the randomisation allocation will be recorded it in the CRF.

7.4. Blinding

Pathologist and laboratory staff processing the operative specimens will be blinded to allocation. However, it is likely that pathologists and laboratory staff will be aware when Patent blue dye has been used, blue staining to specimen, or radioisotope has been used, special handling precautions required.

7.5. Emergency Unblinding

Not applicable

7.6. Baseline data

Baseline data will be collected as per Schedule of Procedures, Appendix 4 and as described below, Section 7.7. Only information directly related to the objectives and outcome measures detailed in the protocol shall be collected.

7.7. Trial assessments

Trial assessments will be performed according to the Schedule of Procedures, Appendix 4

Baseline

- Medical History related to their breast cancer only will be reviewed to confirm eligibility
- Concomitant medications related to their breast cancer only will be reviewed to confirm eligibility
- Height and weight
- The time from injection of ICG to completion of node excision will be recorded.

2 weeks post biopsy

- Record adverse skin reactions i.e seroma formation and cutaneous staining

Follow-up phone call

- Record self-reported skin reactions

Biopsy results

- Staining characteristics of each lymph node will be documented as blue, radioactive, or fluorescent (or a combination).

7.8. Long term follow-up assessments

Not applicable.

7.9. Qualitative assessments

Not applicable.

7.10. Withdrawal criteria

Participants are free to withdraw at any time and are not obliged to give reason(s). The CI, PI or delegate will make a reasonable effort to ascertain the reason(s), both for those who express their right to withdraw and for those lost to follow up, while fully respecting the individual's rights.

The investigator may withdraw a patient at any time if it is in the best interest of the patient and treatment continuation would be detrimental to the patients' wellbeing. A full explanation will be provided. If the trial is being conducted on an intention to treat basis, and the participant has been randomised and given the intervention or standard care, they will be asked to complete trial visits as per the protocol, if the CI considers it appropriate, to allow for an intention to treat analysis - but will be censored in the per-protocol analysis. Participants are free to refuse to do so.

Those withdrawn, including those lost to follow-up, will be identified and a descriptive analysis of them provided, including the reasons for their loss, if known, and its relationship to treatment and outcome.

If a participant withdraws or is withdrawn, they will also have the right to withdraw their data and any research tissue collected. Data collected up to the point of withdrawal will be retained and used for analysis unless a participant expresses their right to withdraw their data.

7.11. Storage and analysis of clinical samples

No research samples will be collected during the trial.

7.12. End of trial

The end of trial at all Sites is defined as last participant last visit. The last visit for each participant will be at the 3-month follow-up phone call. The Sponsor and CI have the right at any time to terminate the trial for clinical or administrative reasons.

The end of the trial will be reported to the Sponsor, REC and NHS R&D Office(s) within 90 days, or 15 days if the trial is terminated prematurely. The CI will ensure that any appropriate follow up is arranged for all participants.

A final clinical trial report will be submitted to the REC and Sponsor within 1 year of the end of the trial.

8. TRIAL TREATMENTS

8.1. Name and description of trial treatments

Participants in each Cohort will be randomised to ICG plus additional tracer (standard care) or ICG alone (intervention).

After randomisation, participants in Cohort 1 will undergo either routine radiocolloid injection 2 hours prior to surgery followed by an injection of 2ml of 0.5% ICG (intradermal/subareolar) at the time of surgery (standard care) or an injection of 2ml of 0.5% ICG alone (intradermal/subareolar) (intervention). The breast will be massaged for 2 minutes before measuring levels of radioactivity in the breast and axilla with a gamma probe and detection of nodes using the florescence PDE camera.

Participants in Cohort 2 will either be injected with 2mls of 2.5% Patent Blue dye followed by an additional 2mls 0.5% ICG (standard care) yielding a total injectate volume (blue dye/ICG) of 3mls or 2mls 0.5% ICG alone (intervention). The fluorescence PDE camera will then be used to observe the subcutaneous lymphatic streams with fluorescent imaging.

Following surgical dissection and excision, staining characteristics of each lymph node will be documented as blue, radioactive, or fluorescent (or combination thereof). The gamma probe and fluorescence PDE camera will be used to check for absence of any residual activity or fluorescence within the axilla. The time from incision to completion of node excision will be recorded in minutes.

8.2. Name and description of intervention and standard care

- Radioisotope Technetium^{99} nanocolloid, 20 MBq
- Patent Blue dye 2.5%
- Indocyanine green (ICG) 0.5%

8.3. Concomitant medication

Details of concomitant medications related to participants' breast cancer only will be recorded on the trial Case Report Form.

8.4. Trial restrictions

All concomitant medications, including COVID-19 vaccine, will be allowed as per standard care. There will be no specific trial restrictions.

8.5. Assessment of compliance with treatment

The time from incision to completion of SLN removal and use of tracers will be recorded in the CRF.

8.6. Pharmacovigilance

As the tracers in this trial are used in standard practice no adverse events will be collected for the trial. The safety profiles of the tracers are well known.

The formation of seromas, evidence of cutaneous staining and any other adverse reactions (AR) to the tracers will be recorded as outcomes and not recorded or reported as adverse events.

8.7. Responsibilities

Chief Investigator (CI) / delegate or independent clinical reviewer:

- Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.

9. STATISTICS AND DATA ANALYSIS

9.1. Sample size calculation

This is a pilot study to investigate the sensitivity of ICG alone as a tracer for visualisation of SLNs. A sample size of 100 is based on the number of cases of SLN biopsy undertaken at the two clinical sites on an annual basis. It is expected that randomisation of 100 patients over a 12/18 month period will collect sufficient data to inform the clinically significant difference and estimated sample size for a larger, multicentre, UK wide randomised controlled trial.

9.2. Statistical analysis plan

A statistical analysis plan will be prepared for analysis of primary and secondary outcomes and will include a plan for handling missing data.

9.2.1. Summary of baseline data and flow of patients

No adjustment for baseline characteristics will be required

9.2.2. Primary outcome analysis

The primary analysis will be performed on the Intention to treat population, for each cohort (Cambridge/ Dundee) separately. As this is not a hypothesis generating trial, all analysis will be exploratory.

Within each group, the number of SLNs for each patient will be recorded numerically in the following categories:

	Study Arm	
Number of SLNs	ICG plus radioisotope or blue dye (standard care)	ICG alone (intervention)
Fluorescence only		
Radioisotope plus fluorescence		
Radioisotope only		
Blue dye plus fluorescence		
Blue dye only		

Pathological exam metastases		

The proportion of patients within each designated category will be recorded and nodes found on pathological examination to contain metastases documented. Comparison is performed on a procedural basis (proportion of patients with a positive node) using logistic regression with patient positive yes/no.

9.2.3. Secondary outcome analysis

The proportion of nodes within each designated category will be recorded and nodes found on pathological examination to contain metastases documented. Comparison is performed on a nodal basis using logistic regression with node positive yes/no. Patient will be used as a random factor in the logistic regression.

ARs will be coded with Medical Dictionary for Regulatory Authorities (MedRA). ARs will be tabulated by Preferred Term and System Organ Class separately and rates of ARs compared between groups.

9.3. Interim analysis

An Interim analysis for futility will be performed after 50 patients have had surgery and node positivity is known. The trial will be terminated if there are no node positive cases amongst the first 50 patients (7 – 10 node positive cases would be expected). As the trial is a pilot study not powered for hypothesis generation, no adjustment of power will be performed.

9.4. Participant population

The Intention to Treat population will consist of all participants who underwent surgery. All analysis will be based on this population.

9.5. Procedure(s) to account for missing or spurious data

As patients are under the care of the surgeons and nodes are extracted during surgery, it is not expected that missing data will occur. Data will be examined for missing data, but no sensitivity analysis accounting for missing data will be performed.

10. DATA MANAGEMENT

10.1. Data collection tools and source document identification

The data management system (DMS) will be Excel as approved by Sponsor and will follow the Sponsor Standard Operating Procedure for Data Management in Clinical Research Studies Using Excel.

Delegated research staff will enter the data required by the protocol into the paper CRFs following training and where recorded on the delegation log. The CRF will not collect more information than is required to meet the aims of the trial and to ensure the eligibility and safety of the participant. The medical notes will act as source data and histology results on individual nodes will be recorded on the CRFs once available (alongside nodal staining characteristics).

The PI may delegate CRF completion but is responsible for completeness, plausibility and consistency of the CRF. Any queries will be resolved by the CI or delegated member of the trial team.

The DMS will be based on the protocol and CRF for the trial and individual requirements of the investigators. The database is managed in line with all applicable principles of medical confidentiality and UK law on data protection, namely, the Data Protection Act 2018 and UK General Data Protection

Regulation (UK GDPR). The Data Controller will be the NHS Tayside and the Data Custodian will be the CI.

Delegated research staff will enter data collected on the CRF into the DMS following training and where recorded on the trial delegation log.

CRFs completed locally on the Cambridge cohort of patients will be scanned and sent to Dundee for centralised entry and recording. Data from both Dundee and Cambridge will be entered onto a combined spreadsheet.

All electronic data will be stored on a secure NHS Taysideor cloud-based servers which have restricted access and have disaster recovery systems in place.

10.2. Access to Data

Hard copies of the CRF will stored in the ISF in a secure office.

Access to the DMS will be restricted to members of the trial team trained and identified in the trial delegation log.

The CI, PIs and all institutions involved in the trial will permit trial related monitoring, audits, REC review, and regulatory inspection. In the event of an audit, the CI will allow the Sponsor, representatives of the Sponsor or regulatory authorities direct access to all trial records and source documentation.

10.3. Archiving

All trial documentation, electronic and paper, will be kept for 5 years. Medical case notes will be maintained in compliance with local NHS Policy on Retention of Medical Case notes.

11. MONITORING, AUDIT & INSPECTION

11.1. Monitoring

The study may be selected for audit and/or monitoring by the Sponsor.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Research Ethics Committee (REC) review & reports

Before the start of the trial, approval will be sought from a REC for the trial protocol, ICF and other relevant documents e.g. GP information letters

Substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion for the trial (note that amendments will need to be reviewed and accepted by NHS R&D departments before they can be implemented in practice at site)

All correspondence with the REC will be retained in the TMF/ISF

An annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended

It is the Chief Investigator’s responsibility to produce the annual reports as required.

The Chief Investigator will notify the REC of the end of the trial

If the trial is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination

Within one year after the end of the trial, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC

12.2. Peer review

This project has been peer reviewed by the Association of Breast Surgeons, the NHS Tayside Breast Unit Clinical Governance Meeting

12.3. Public and Patient Involvement

A previous research proposal involving use of ICG for SLN biopsy was discussed at a local research meeting attended by a patient representative. They read through the protocol in detail and considered that use of ICG instead of conventional tracer agents and in particular radioisotopes would confer benefit in terms of cost-effectiveness and factors such as avoidance of separate hospital visits in those units where radioisotope injection is performed the day before surgery or at a geographically different facility. It was also pointed out that patients would experience less discomfort associated with tracer injection as the patient is already anaesthetised when injected with fluorescent tracer. During the conduct of ICG-10 feasibility study that involved addition of ICG as a third tracer for SLN localization, patients were provided with detailed information leaflets and fully informed consent obtained. Rates of acceptance within this study exceeded 95% and patients were very keen to participate in a study which might eventually lead to abandonment of radioactive tracers and blue dye which each have their own problems.

The PIS and ICF have been reviewed by Patient and Public Involvement representatives.

12.4. Regulatory Compliance

The trial will not commence until a favourable REC opinion is in place. Before enrolling patients into the study, the Chief Investigator/Principal Investigator or delegate will ensure that appropriate approvals from participating organisations are in place.

For any amendment to the trial, the CI, PI or delegate, in agreement with the sponsor, will submit information to the appropriate body in order for them to issue approval for the amendment. The CI, PI or delegate will work with sites (NHS R&D departments at sites as well as the trial delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the trial as amended.

As the administration of radioisotopes is not additional to normal standard care, Administration of Radioactive Substances Advisory Committee certificates are not required.

12.5. Protocol compliance

The CI will not implement any breach of the protocol without agreement from the Sponsor, except where necessary to eliminate an immediate hazard to trial participants.

In the event that there is a breach of the protocol, the nature of and reasons for the breach will be recorded in the CRF/TMF and documented in the trial Breach Log.

It is Sponsor policy that waivers to the Protocol will not be approved.

12.6. Notification of Serious Breaches to GCP and/or the protocol

In the event that there is a breach of protocol, the nature of and reasons for the breach will documented in the trial Breach Log.

If a breach of the protocol or GCP is suspected, this will be reported to the Sponsor immediately using the TASC Breach Reporting Form and documented in the trial Breach Log.

If a breach necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC, and NHS R&D for review and approvals as appropriate.

12.7. Data protection and patient confidentiality

The CI and trial staff will comply with the requirements of the Data Protection Act 2018 and UK General Data Protection Regulation (UK GDPR) or any subsequent amendment or replacement thereof with regard to the collection, storage, processing and disclosure of personal information and will uphold the Directive's core principles.

The CI and trial staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or equivalent.

All trial records and data will be managed in a manner designed to maintain participant confidentiality. All records, electronic or paper, will be kept in a secure storage area with access limited to appropriate trial staff only. Computers used to collate data will have limited access measures via usernames and passwords.

Personal clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or regulatory authorities.

The CI and trial staff will not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial. Prior written agreement from the Sponsor will be required for the disclosure of any said confidential information to other parties.

Access to collated participant data will be restricted to the CI and appropriate delegated trial staff.

Where data requires to be transferred, an appropriate Data Transfer Agreement will be put in place.

Published results will not contain any personal data that could allow identification of individual participants.

12.8. Financial and other competing interests for the Chief Investigator, PIs at each site and committee members for the overall trial management

There are no competing interests that might influence the trial design, conduct or reporting.

12.9. Indemnity

Tayside Health Board is sponsoring the trial.

Insurance: Tayside Health Board will maintain its membership of the Clinical Negligence and Other Risks Insurance Scheme (CNORIS) which covers the legal liability of Tayside in relation to the trial.

Where the trial involves University of Dundee staff undertaking clinical research on NHS participants, such staff will hold honorary contracts with Tayside Health Board which means they will have cover under Tayside's membership of the CNORIS scheme.

Indemnity: The Sponsor does not provide trial participants with indemnity in relation to participation in the Trial but has insurance for legal liability as described above.

Where other UK NHS organisations are participating as trial sites, those other UK NHS organisations will maintain membership of a scheme similar to CNORIS.

12.10. Amendments

The CI will seek Sponsor approval for any amendments to the Protocol or other approved trial documents. Amendments to the protocol or other trial documents will not be implemented without approval from the Sponsor and subsequent approval from the appropriate REC and NHS R&D Office(s).

12.11. Post-trial care

As this trial is comparing the standard tracers used for visualising the lymphatic system and nodes, it does not involve any changes to treatment expected under standard care. Participants will be treated and followed up as per the local breast cancer surgery service.

12.12. Access to the final trial dataset

The CI and trial statistician will have access to the final trial dataset. Access to the final trial dataset to others will be approved by the CI.

13. DISSEMINATION POLICY

13.1. Dissemination policy

The trial will be registered on International Standard Randomised Controlled Trials (ISRCTN) register. Details of the trial will be published on ISRCTN no later than 12 months after the end of the trial. The report will be made available to the funder. The report can be used for publication and presentation at scientific meetings. Trial investigators have the right to publish orally or in writing the results of the trial. The criteria for authorship will follow the criteria of the International Committee of Medical Journals.

Publications will be reviewed according to the agreed contractual terms but will not restrict the general rights outlined above for the Investigators to publish the results of the trial.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion). A newsletter giving a summary of the results of the trial will be made available to participants.

13.2. Authorship eligibility guidelines and any intended use of professional writers

The data arising from this trial resides with the trial team and ownership with NHS Tayside. On completion of the trial, the trial data will be analysed and tabulated, and a clinical trial final report will be prepared.

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15. APPENDICIES

15.1. Appendix 1 - Risk

Risks associated with trial interventions

☒ A ≡ Comparable to the risk of standard medical care

☐ B ≡ Somewhat higher than the risk of standard medical care

☐ C ≡ Markedly higher than the risk of standard medical care

Justification:

Standard SLN detection techniques involve the use of blue dye or radioisotopes. ICG fluoresce detection is routinely used in combination with these standard techniques. Participants in this this study will be in two cohorts. Participants in Cohort 1 will be randomised to either ICG alone (intervention) or ICG plus radioisotope (standard care). Participants in Cohort 2 will be randomised to ICG alone intervention) or ICG plus blue dye (standard care).

What are the key risks related to therapeutic interventions you plan to monitor in this trial?		How will these risks be minimised?		
Intervention	Body system/Hazard	Activity	Frequency	Comments
Possibility of the ICG alone not identifying SLN	Breast/axilla	Where no SLNs are identified by the ICG the surgeon will randomly sample axillary nodes. This is normal practice when this occurs during standard care.	At time of surgery	There is a small chance that this may cause an increase in seroma formation and the possibility of the development of lymphoedema

Outline any other processes that have been put in place to mitigate risks to participant safety (e.g. DMC, independent data review, etc.)

None required

Outline any processes (e.g. IMP labelling +/- accountability +/- trial specific temperature monitoring) that have been simplified based on the risk adapted approach.

None required

15.2. Appendix 2 - Trial management / responsibilities

Responsibilities will be detailed the co-sponsorship and model trial agreements.

15.2.1. Patient registration/randomisation procedure

An independent statistician will generate a randomisation list for both sites. The allocation arm for each participant will be placed into sealed double envelopes with the participant specific identification number on the outside; each envelope will only contact the allocation for one participant. Data management

15.2.2. Data Management

The trial data management system will be Excel. Local sites staff will be expected to compete the paper CRF. The CRFs from Cambridge will be scanned and transferred securely to Tayside for entry into Excel. All data on the CRF should be entered into the Excel spreadsheet within 21 days of the last data collection point for that participant. The PI or delegate is reasonable for resolving all data queries. Return of queries should be within 2 weeks.

15.2.3. Preparation and submission of amendments

The CI is responsible for the submission of amendments.

15.2.4. Preparation and submission of Annual Safety Report/Annual

The CI is responsible for the submission of annual reports to REC, cc to sponsor.

15.2.5. Data protection/confidentiality

The CI and trial staff will comply with the requirements of the Data Protection Act 2018 and UK General Data Protection Regulation (UK GDPR) and the Data Protection Act 2018 or any subsequent amendment or replacement thereof with regard to the collection, storage, processing and disclosure of personal data and will uphold the Principles of GDPR in Article 5.

The CI and trial staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or local equivalent.

15.2.6. Trial documentation and archiving

Archiving trial site data will be the responsibility of individual sites. Payment for archiving will be provided as per site agreement.

15.3. Appendix 3 – Authorisation of participating sites

15.3.1. Required documentation

The following data should be made available to the CI prior to site initiation:

- PI CV, signed and dated
- PI GCP certificate
- Protocol signature page, signed and dated by PI
- Copy of signed Participating Site Agreement
- Copy of R&D confirmation of capacity and capability

The following data should be made available and held within the ISF/PSF prior to site initiation:

- CV, signed and dated for all trial staff listed on Delegation Log
- GCP certificate for all trial staff listed on Delegation Log

15.3.2. Procedure for initiating/opening a new site

Site Initiation may be carried out remotely or face to face.

Site Initiation will be performed by TCTU Trial Management Team.

15.3.3. Principal Investigator responsibilities

The PI's legal responsibilities will be listed in the participating site agreement a summary is given below:

- Attendance at the initiation teleconference,
- Training of new members of trial staff in the protocol and its procedures,
- Ensuring that the ISF is accurately maintained,
- Dissemination of important safety or trial related information to all stakeholders within their site
- Safety reporting within the required timelines
- Ensuring data entry to CRF and responses to data clarification queries are completed within the required timelines.
- Certify data entered on CRF is correct and complete.
- Ensure CRFs are scanned and transferred to Tayside for data entry.
- Archiving of site trial data.

15.4. Appendix 4 – Schedule of Procedures

	Visit 1 Screening	**Visit 2 Cambridge only Consent**	**Visit 3 Tayside only Baseline**	**Visit 4 Follow-up**	**Visit 5 Follow-up**	**As results are available**
Type of Visit	**Routine pre-biopsy visit**	**RN phone call**	**Routine biopsy visit**	**Routine visit**	**RN Phone call**	
Timeline	**Day -21 to 0**	**Day -20 to 0**	**Day 1**	**2 weeks (+/- 5 days)**	**3 months (+/- 7 days)**	
Eligibility	X					
Provide PIS	X					
Informed Consent		X	X (Tayside only)			
Record Medical History			X			
Record Concomitant Medications			X	X	X	
Inclusion/exclusion		X	X (Tayside only)			
Randomisation		X	X (Tayside only)			
Record results of physical examination			X			
Record seroma formation and cutaneous staining				X	X	
Staining characteristics of SLN						X

15.5. Appendix 5 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
N/A	1		As section VI	N/A