**Indocyanine green compared with technetium-99m for sentinel lymph node biopsy in breast cancer: A prospective trial**

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| **Protocol Number** | HREC X21-0001 |
| **Coordinating Principal/ Chief Investigator** | A/Prof Sanjay Warrier |
| **Protocol author** | Dr Chu Nguyen |
| **Signature:** C.N | **Date:** 24/06/2021 |
| **Co-investigators** | A/Prof Carlos Pulitano, Dr Chu Nguyen |
| **Protocol Version Number** | 4 |
| **Protocol Date** | 24/06/2021 |

The study is being conducted by Dr Chu Nguyen as part of the requirements for a PhD degree under the supervision of A/Prof Sanjay Warrier and A/Prof Carlo Pulitano.

**Ethics Statement:**

The study will be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* (2007) ([Link to National Statement](https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018)) , the *CPMP/ICH Note for Guidance on Good Clinical Practice* ([Link to CPMP/ICH](https://www.tga.gov.au/publication/note-guidance-good-clinical-practice-july-2000) ) and consistent with the principles that have their origin in the Declaration of Helsinki. Compliance with these standards provides assurance that the rights, safety and well-being of trial participants are respected.

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| **Protocol Summary** | |
| **Protocol Title** | Indocyanine green compared with technetium-99m for sentinel lymph node biopsy in breast cancer: A prospective trial |
| **Protocol version** | 4 |
| **Objectives** | Primary objective: To compare the equivalency of indocyanine green (ICG) to technetium-99m (99mTc) for axillary sentinel lymph node (SLN) lymphatic mapping in patients with early-stage breast cancer in terms of number of nodes identified and the identification of pathologically positive nodes.  Secondary objective: To compare the surgeon’s reported ease of identifying sentinel lymph nodes with ICG to 99mTc, and to identify any complications related to its use. |
| **Study design** | Prospective cohort study. |
| **Planned sample size** | 93 patients. |
| **Selection criteria** | Patients with clinically node-negative early breast cancer undergoing sentinel lymph node biopsy. |
| **Study Procedure** | Breast cancer patients will be prospectively enrolled and undergo sentinel lymph node biopsy using the standard-of-care procedure with the radioisotope, technetium (99mTc), as well as indocyanine green (ICG) using a near infrared camera (SPY-PHI fluorescence imaging technology).  Patients will receive subdermal/ peri-tumour injection of 99mTc-labeled tracer for lymphoscintigraphy preoperatively followed by intraoperative injection of ICG for fluorescence detection of sentinel lymph nodes (SLN). Sentinel lymph nodes first identified by the fluorescence method (ICG-positive) will be removed and a gamma-detecting probe then used to determine whether ICG-positive SLNs are hot (99mTc -positive) and to identify and remove any 99mTc -positive (ICG-negative) SLNs remaining in the axilla. To assess the need for 99mTc to localize the SLN, the gamma- detecting probe will be utilised if no lymph nodes are initially localised with ICG.  A comparison of the detection rate and diagnostic accuracy of the two methods will be performed to detect equivalency of the two methods. |
| **Statistical considerations** | 224 sentinel lymph nodes need to be examined to demonstrate equivalence between the two methods with 80% power and type I error (a) of 5%. Tests for equivalence and results will be presented with a 2-sided 95% confidence interval compared with 5% equivalence region. McNemar test will be used to compare the difference in detection rate between the 2 methods for pathologically positive axillary SLNs. Statistical analysis will be performed with RStudio, version 1.4.1106. |
| **Time Period of Data Collection** | June 2021 to February 2022. |
| **Duration of the Study** | 9 months. |
| **Funding** | Chris O’Brien Lifehouse |
| **Sponsor** | Chris O’Brien Lifehouse |

# BACKGROUND AND INTRODUCTION

### 1.1. DISEASE/PROPOSED INTERVENTION BACKGROUND

The aim of this study is to prospectively compare the equivalency of indocyanine green (ICG) to technetium-99m (99mTc) for axillary sentinel lymph node (SLN) lymphatic mapping in patients with early-stage breast cancer in terms of number of nodes identified and the identification of pathologically positive nodes. This is to determine whether ICG can be effectively used alone to identify the SLN.

Lymphatic mapping and axillary sentinel lymph node biopsy (SLNB) are the standard procedures for surgical staging of patients with early-stage breast cancer. Sentinel lymph node biopsy is a safe procedure that provides a reliable indicator of the metastatic status of the axilla. Nodal status is a crucial determinant of prognosis and determines any adjuvant therapies that may be required to treat the cancer.1, 2 Sentinel lymph node biopsy has reduced the need for traditional axillary dissection in most patients.3 During SLNB, the SLNs are identified through lymphatic mapping and are then biopsied to assess whether metastatic spread has occurred. This involves injecting a tracer around the tumour and following the drainage pathway to identify the first nodes that have ‘taken-up’ the tracer. These nodes are the ‘sentinel’ lymph nodes.2

Gold standard lymphatic mapping involves using a combination of blue dye (BD; patent blue, methylene blue, or isosulphan blue) and radioactive colloid labelled with technetium-99 (99mTc). These involve subdermal injections into the peri-areolar or peri-tumour area. Sentinel nodes are defined as axillary lymph nodes in which there is accumulation of one or more of these tracers or dyes at higher than defined thresholds.4

Blue-dye injections are conveniently performed during the operation, but it’s disadvantages include skin necrosis, anaphylaxis and lower sensitivity (91%) when used alone.5,6 The gold standard dual technique of BD and 99mTc has a SLN detection rate of 96.7% (95% confidence interval [CI] 94.3%, 99.1%).7 This approach is highly sensitive with low false negative rates.5 The use of BD alone has a SLNB detection rate of 86.8% (95% CI: 82.7%, 91.0%).The use of 99mTc alone has a SLN detection rate of 96.5% (95% CI 95.2%, 97.9%)..7 It however has many disadvantages, including radiation exposure, need for nuclear medicine access with special licencing and hospital infrastructure for safe use and disposal, patient inconvenience with injections done prior to the surgery, and if used in isolation there is lack of visual cues to identify lymph nodes.8-11 These limitations with both BD and 99mTc mapping have led to the development of new techniques to in SLNB, such as fluorescence imaging.7, 12, 13

Indocyanine green (ICG) was first developed for infrared photography. Since the 1970s it has been used in ophthalmology to assess retinal and choroidal circulation.14 Indocyanine green is currently Food and Drug Administration (FDA) approved for use with medical applications.15-17 In Australia, it can be approved by the Therapeutic Goods Administration (TGA) for intraoperative diagnostic use in patients as a Special Access Scheme Category C drug. It involves real-time fluorescence imaging utilising a near-infrared (NIR) camera after subdermal administration of the fluorophore, indocyanine green, which is a tricarbocyanine iodide dye.18 Indocyanine green has an excellent safety profile with a low incidence of adverse events (1 out of 42,000 patients) and short plasma half-life of three to four minutes.19-21

Indocyanine green has been used for SLNB in gastrointestinal, melanoma and breast cancer.22-24 Use of ICG for lymphatic mapping and SLN identification has advantages over the use of conventional techniques, including that the injection can be given intraoperatively, there is instant visualization of lymphatic anatomy and flow, and it is low cost.25 A recent meta-analysis reported that ICG SLNB was a safe and effective alternative to BD or 99mTc. Sentinel lymph node biopsy with ICG was not inferior to the dual technique or 99mTc alone, but was superior to BD. Indocyanine green SLNB also does not have the risks associated with radioisotopes, skin tattooing and hypersensitivity reactions.12

### RATIONALE FOR PERFORMING THE STUDY

There are significant advantages to use of ICG in SLNB compared to the 99mTc technique. Indocyanine green can provide dynamic real-time visualization of lymphatic anatomy as ICG travels from the injection site towards the SLN. This is viewed on a monitor through the NIR camera. Technetium-99m is detected by audio cues with a gamma-detecting probe. This makes it static in feedback as it only provides signal where sufficient accumulation of the tracer has occurred in SLNs.26 The administration of ICG is also more convenient as a subdermal injection given intraoperatively. Technetium-99m requires a nuclear medicine procedure up to one day prior to the operation, resulting in more hospital resources and increased time burden on the patient.26, 27

The current gold standard for axillary sentinel lymph node biopsy is the dual technique of blue dye and radioisotope. Singe technique use of either BD or 99mTc is still considered to be standard-of-care, especially at institutions that do not have access to infrastructure. At our institution, blue dye is no longer used given the high anaphylaxis rate. The current standard-of-care here is use of 99mTc alone.

The dual technique of BD and 99mTc has been shown to have an identification rate of 96.7% (95% CI: 94.3%, 99.1%) and false-negative rate of 5.5% (95% CI: 0.9%, 10.2%). Despite the effectiveness of the dual technique, use of both BD and 99mTc pose challenges for smaller institutions that lack the require infrastructure.28

The use of 99mTc tracer alone has been shown to have a pooled 96.5% (95% CI: 95.2%, 97.9%) identification rate with false-negative rate of 2.6% (95% CI: 0.7%, 4.6%) from recent multi-centre meta-analyses. It is clinically effective but handling of isotopes requires availability, infrastructure for disposal of the isotopes, training of the staff, and legislative requirements. The use of BD alone has an identification rate of 86.8% (95% CI: 82.7%, 91.0%) and false-negative rate of 18.4% (95% CI: 11.9%, 24.9%).28

ICG alone has an identification rate of 97.9% (95% CI: 96.9%, 98.9%) and false-negative rate of 0.6% (95% CI: -0.3%, 1.5%).28 ICG fluorescence has been demonstrated to be 8.89 times more likely to identify a sentinel lymph node than BD alone, and 4.22 times more likely than the dual technique. When ICG is compared to 99mTc alone, there is no statistically significant difference in sentinel lymph node identification rates. SLNB with ICG offer a safe and effective alternative to either BD or 99mTc. In summary, SLNB with ICG is non-inferior to the dual technique and 99mTc alone, while being superior to BD.7, 12, 13, 28

The proposed trial will use the dual technique of ICG and radioisotope. To our knowledge there has been no published studies on ICG use for SLNB in Australia. Recent studies from Europe and Japan have demonstrated comparable efficacy between the number of SLNs identified between ICG and 99mTc in breast cancer.13, 24, 29-31 In order for us to consider adopting this technology into routine clinical practice, it’s use needs to be validated in an Australian population. The results of recent meta-analyses suggest that ICG is not inferior to the dual technique or either technique used in isolation.12, 13, 28 There are however no RCTs comparing ICG with the dual technique or 99mTc alone. This is likely because ICG is still a relatively novel technique and so the safest option is to use combined techniques to minimise the risk of missing SLNs. At this stage, a safe approach would be for us to perform a prospective cohort study using a combination of ICG and 99mTc. If the results validate that ICG is comparatively effective and safe, we could potentially proceed to a RCT in future to randomize patients to ICG alone and 99mTc alone.

If ICG is confirmed to be as effective as 99mTc then this is a crucial finding for clinical practice because ICG has advantages over the standard 99mTc technique. In particular, ICG allows for direct implementation in theatre with no prior preparation or need to involve nuclear medicine which is especially beneficial for hospitals without access to nuclear medicine.

# 2. HYPOTHESIS

Indocyanine green with fluorescence imaging for axillary sentinel lymph node identification is equivalent to the single technique of technetium-99m. Use of indocyanine green has comparable efficacy and improved safety profile, including lesser burden on hospital infrastructure.

# 3. STUDY OBJECTIVES / AIMS

### 3.1. PRIMARY OBJECTIVES

To compare the equivalency of indocyanine green (ICG) to technetium-99m (99mTc) for axillary sentinel lymph node (SLN) lymphatic mapping in patients with early-stage breast cancer in terms of the number of nodes identified and the identification of pathologically positive nodes.

### 3.2. SECONDARY OBJECTIVES

To compare the surgeon’s reported ease of identifying SLN with ICG to 99mTc, and to identify any complications related to its use.

# 4. STUDY DESIGN

Training of all study personnel in the protocol will be undertaken prior to the trial commencing.

### 4.1. DESIGN / STUDY TYPE

Prospective cohort study.

### 4.2. EXPECTED PARTICIPANT NUMBERS

93 patients based on sample size estimates detailed in Section 6.2. Competency with a surgical technique will be defined as at least 45 cases. The two major phases of a learning curve are the initial and plateau phases. The initial phase involves the number of times a task needs to be performed before a surgeon learns it. This phase classically shows a sharp rise that equates to improvement in performing the task with repetition. The plateau phase is reached when improvement slows. It has been shown that rates of complications significantly decreased with increasing surgeon experience as defined by the number of cases and defined time periods.32,33

There are no trials to date that have investigated the learning curve of ICG in SLNB. ICG provides real-time visualisation of lymphatics and could be hypothesized to be easy to use compared to blue dye and 99mTc. A previous study demonstrated that surgeons achieve a satisfactory localization rate and acceptable false-negative rate after 40 SNLBs using standard-of-care tracers. This was an evaluation based on the landmark multicenter randomized trial, ALMANC trial.34

Our proposed trial would allow us to assess the learning curve of ICG in SLNB as all the surgeons at our institution are already proficient at SLNB using the dual technique and 99mTc alone. Results from the analysis of ICG in SLNB learning curves would be related to the imaging technology rather than the SLNB procedure itself. Our definition of progress along the learning curve would be that surgeons must achieve a localization rate of 90% or more and a false-negative rate of 5%.

### 4.3. TIME PERIOD OF THE STUDY

The trial will start from June 2021 to February 2022. It is estimated that 9 months will be enough time to produce at least 93 cases.

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| **Task** | **Start Date** | **End Date** |
| **Ethics Submission** | April 2021 | May 2021 |
| **Ethics Review and Approval** | May 2021 | May 2021 |
| **Recruitment** | June 2021 | June 2021 |
| **Collection of data** | June 2021 | February 2022 |
| **Analysis of Data** | February 2022 | February 2022 |
| **Publication Draft** | March 2022 | March 2022 |
| **Submission of Publications and Final Reports** | April 2022 | April 2022 |

### 4.4. ENDPOINTS

PRIMARY ENDPOINTS

* Number of sentinel lymph nodes identified with ICG and 99mTc methods.
* Number of metastatic nodes positive and negative for ICG and 99mTc methods.

SECONDARY ENDPOINTS

* The surgeon’s reported ease of identifying SLNs with ICG compared to 99mTc.
* To identify any complications related to ICG and 99mTc.

### 4.5. CENTRE

There will be only 1 study site.

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| **Site Name** | Chris O’Brien Lifehouse |
| **Site Contact/Investigator** | A/Prof Sanjay Warrier, Dr Chu Nguyen |
| **Public Health Organisation (PHO)** | No |
| **If a non-PHO is an External Entity Agreement (EEA) in place?** | Yes |
| **Study Procedures** | Recruitment/ data collection / data analysis / data storage |

# 5. STUDY PARTICIPANTS

### 5.1. INCLUSION CRITERIA

### - Participant has clinically node-negative early breast cancer undergoing sentinel lymph node biopsy

### - Female, age 18 years or older

### - Participant understands the study procedures and can provide informed consent to participate in the study

### 5.2. EXCLUSION CRITERIA

### - Participant does not have clinically node-negative early breast cancer (tumour size >3 cm +/- clinically positive lymph nodes)

### - Participant has known contraindication to ICG injection, i.e., previous reaction to ICG

### - Participant has Iodine allergy

### - Participant has chronic kidney disease stage 3, 4, or 5

### - Participant is pregnant

### - Refusal to consent to participation in the study

5.3 Key Elements of Recruitment (as per NS)

Participants will only be recruited if they meet the inclusion and exclusion criteria. The current gold standard for axillary sentinel lymph node biopsy is the dual technique of blue dye and radioisotope. At Chris O’Brien Lifehouse, blue dye is no longer used given the high anaphylaxis rate. The proposed trial will use the dual technique of ICG and radioisotope.

* Who will be recruited?

### Patients who are being scheduled for sentinel lymph node biopsy for clinically node-negative early breast cancer.

* How will participants be identified and recruited?

The Surgeons, Fellows and Registrars will have contact with the patients who have been referred to the Surgeon’s rooms or the Hospital’s Breast Clinic by GPs or specialists or from the BreastScreen NSW Program. This is all part of routine standard-of-care. Recruitment and consent will be carried out by a medical officer who is a researcher and independent from the attending medical team. If the patient meets the inclusion and exclusion criteria for the study, they will be consented for participation. Patients with a new diagnosis of breast cancer have multiple outpatient clinic visits prior to consent for their operation. The initial visit mostly provides them with the details of their cancer, any further tests required such as imaging, and the potential treatment they may require including adjuvant therapy and surgery. Their case may then be discussed at a Multidisciplinary Meeting (MDT) to come to an agreement about the most appropriate treatment. They then have further outpatient clinic visits to discuss these treatments and the consent for an operation, if it is indicated, finally takes place. It is at this subsequent visit that information about participation in the trial will be provided to the patient.

* Will the potential participants be screened?

Patients will be screened based on inclusion and exclusion criteria during the initial consultation.

* What is the impact of any relationship between researchers and potential participants on recruitment?

All participants will be receiving routine standard-of-care for management of their breast cancer whether or not they choose to participate in the study.

* How will the recruitment strategy facilitate obtaining the consent of participants?

Patients will be reviewed by the Consultant, Fellow or Registrar as usual during the initial pre

operative visit. A medical officer who is a researcher and independent from the attending medical

team will be invite the patient to participate in the study during this visit and they will be provided with

at least 1 week to consider participation in the study.

* How will the recruitment strategy ensure that participants can make an informed decision about participation?

The consent will be carried out by a medical officer who is a researcher and independent from the attending medical team who will have adequate training to consent patients. The consent will be obtained in a way that the patient is informed about the nature of the study, risks and benefits, plans for use of the data for analysis and dissemination at scientific conferences and publication in a journal. An interpreter will be arranged if required. The consent will be specific for the additional imaging technique used in the study. The patient will not be coerced. The patient will have at least 1 week to consider participation in the trial. The patient’s participation in the trial will be revisited during their preadmissions visit. This will have given them at least a week to consider participation as cancer surgery often is booked on the waitlist as “Category A” urgency (within 30 days). Consent for participation in the trial will take place during this visit. This will be carried out by a medical officer who is a researcher and independent from the attending medical team.

* Are there any risks associated with the recruitment strategy for potential participants or for the viability of the project?

No risks identified.

5.4 CONFOUNDERS

Potential confounders include:

- Baseline demographics: age, tumour type, previous breast or axilla surgery and obesity.

- Surgical factors: surgeon experience with ICG technology.

5.5 STUDY LIMITATIONS

A limitation will be that the study is that it is not randomized. This is because, the safety and efficacy of the technique needs to be validated first. This involves using the technique in combination with standard-of-care 99mTc to avoid missing any sentinel lymph nodes.

# 6. STUDY PROCEDURES

### 6.1. STUDY FLOW CHART

**Enrolment**

Setting: 1 centre

Population: Patients with clinically node-negative early breast cancer undergoing sentinel lymph node biopsy

Inclusion criteria: Female, age 18 years or older; Being scheduled for clinically node-negative early breast cancer undergoing sentinel lymph node biopsy; Participant can provide informed consent to participate.

Exclusion criteria: Participant does not have clinically node-negative early breast cancer (tumour size >3 cm +/- clinically positive lymph nodes; Contraindication to ICG, i.e., previous reaction to ICG; Iodine allergy; Chronic kidney disease stage 3, 4, or 5; Pregnant; Refusal to consent to participate.

**Baseline data collection**

Demographics, tumour characteristics, previous surgery, obesity

**Surgery**

Standard-of-care sentinel lymph node biopsy with the addition of indocyanine green for sentinel lymph node identification

7 days post-operative followup

Clinical assessment for complications

Histopathology results of sentinel lymph node biopsy reviewed

30 days post-operative followup

Clinical assessment for complications

### 6.2. INVESTIGATION PLAN

***Summary***

Patients with clinically node-negative early breast cancer undergoing sentinel lymph node biopsy (SLNB) will be prospectively enrolled. All the patients will undergo sentinel lymph node (SLN) detection using the standard-of-care procedure with the radioisotope technetium-99m (99mTc). In addition, indocyanine green (ICG) using a near-infrared (NIR) camera (SPY-PHI fluorescence imaging technology) will be used.

Patients will receive subdermal/ peri-tumour injection of 99mTc-labeled tracer for lymphoscintigraphy preoperatively followed by intraoperative injection of ICG for fluorescence detection of sentinel lymph nodes (SLN). Sentinel lymph nodes first identified by the fluorescence method (ICG-positive) will be removed and a gamma-detecting probe then used to determine whether ICG-positive SLNs are hot (99mTc -positive) and to identify and remove any 99mTc -positive (ICG-negative) SLNs remaining in the axilla. To assess the need for 99mTc to localize the SLN, the gamma-detecting probe will be utilised if no lymph nodes are initially localised with ICG.

A comparison of the detection rate and diagnostic accuracy of the two methods will be performed to detect equivalency of the two methods.

**Methodology**

***Study design***

This will be a single-centre prospective cohort study. The inclusion criteria are female, age 18 years or older, with early breast cancer confirmed by core or fine needle biopsy, and a clinically negative axilla, and scheduled for SLNB. Exclusion criteria are patients with tumour size >3 cm, clinically positive lymph nodes, known contraindication to ICG, i.e., previous reaction to ICG, iodine allergy, chronic kidney disease stage 3, 4, or 5, or pregnancy. Patients meeting the inclusion criteria will undergo standard-of-care SLNB with the addition of ICG use. Preoperative data, including the patients’ demographics, tumour characteristics, previous breast or axilla surgery and body mass index (BMI) will be recorded.

***Surgical technique***

Patients eligible for SLNB will undergo standard-of-care preoperative lymphoscintigraphy with subdermal/ peri-tumour injection of 99mTc the day prior to surgery. At the time of surgery after anaesthesia and immediately before the operation, 0.5 mL of Infracyanine® 25mg/10mL (SERB, Paris, France) will be injected subdermally into the peri-areolar area of the breast. Movement of ICG in the lymph ducts will be facilitated by manual massage.

Operating room lights will be turned off to avoid any potential interference of ambient light with detection of ICG fluorescence. Fluorescence of ICG will be immediately elicited and detected by the near-infrared (NIR) camera (SPY-PHI, Novadaq Technologies Inc., Mississauga, Canada). The lymphatic drainage will be visualized in real time on a monitor. The fluorescence will be followed from the site of injection towards the axilla, and where the fluorescence disappears into the axilla, an incision is made to commence the biopsy.

The fluorescent lymphatic channels will then be dissected and followed to the first ICG avid lymph node with assistance from the NIR camera. The lymphatic channels will be clipped and ligated to avoid ICG leakage into the dissection field which can distort visualisation of lymphatic anatomy. Fluorescent lymph nodes (ICG-positive) will then be localized and excised. These excised ICG-positive nodes will then be tested for radioactivity using a gamma-detecting probe and classified as hot (99mTc-positive) or cold (99mTc-negative). Sentinel lymph node removal will continue until no residual fluorescence is visible in the axilla.

Finally, the axilla will be inspected with the gamma-detecting probe to determine whether any radioactivity is left. If there is significant residual radioactivity the hot spot (considered to be a 99mTc-positive SLN) will be removed and examined. The number of sentinel nodes (ICG-positive, 99mTc-positive, or both) removed from each patient will be documented. With regard to radioactivity, a SLN will be defined as any node that when measured ex vivo will have a gamma count ratio, compared to the background axilla, of 10:1. All SLNs will be sent for histopathology analysis.

Sentinel lymph nodes will be compared to identify concordance among those identified as fluorescent, radioactive, and both. The total number of SLNs identified by ICG, 99mTc, or both, will be recorded.

***Outcomes***

Demographic information will be collected including tumour type, previous breast or axilla surgery and obesity.Intraoperative information will be collected including the number of nodes identified with ICG and 99mTc, which technique allowed for first detection of the lymph node, the surgeon’s reported ease of detection with ICG and 99mTc, and operation duration.Postoperative information will be collected including hospital admission duration, complications, the number of pathologically positive lymph nodes on histopathology, the need for any axillary treatment (none, radiotherapy or axillary dissection), and followup visit information at 7 and 30 days postoperative.

***Statistical analysis***

Data will be described using means or medians where appropriate, ranges for continuous variables, and counts and percentages for categorical variables.

Sample size estimates were calculated to evaluate the equivalency of ICG to 99mTc in terms of detection rate of sentinel lymph nodes. They are based on a detection rate with 99mTc of 96.5%. A δ = 5% difference in detection rate was of no clinical significance. Using a true detection rate, θ0 = 97.9%, at α = 0.05, the sample size to yield 80% power (β=0.2) is n = 224 sentinel lymph nodes. The mean number of sentinel lymph nodes removed based on the literature was taken as 2.4, equating to at least 93 patients needed for this trial.35

The null hypothesis for equivalence of ICG and 99mTc SLN mapping techniques will be the difference between the proportion of SLNs detected by each method that lie outside the margin (-5%, 5%). Tests for equivalence will be based on analysing clustered paired binary data and results presented by a 2-sided 95% confidence interval compared with 5% equivalence region. McNemar test will be used to compare the difference in detection rate between the 2 methods for pathologically positive axillary SLNs.29, 36 Statistical analysis will be performed with RStudio, version 1.4.1106.

### 

### 6.3. STUDY PROCEDURE RISKS

There is a small risk of an adverse reaction to indocyanine green. It has an excellent safety profile with a low incidence of adverse events (1 out of 42,000 patients). Most reported cases of adverse events have been mild and transient.20, 21

### 6.4. PARTICIPANT RECRUITMENT AND SCREENING

Recruitment by the surgical team inherently results in the potential for perceived coercion. To avoid this there will be separation of treating medical officers from research trial personnel undertaking enrolment of participants. Recruitment and consent will be carried out by a medical officer who is a researcher and independent from the attending medical team.

|  |  |
| --- | --- |
| **Will participants be screened?** | Yes |
| **If yes, what data will be collected?** | Inclusion and exclusion criteria will be documented. If the patient is not eligible then the data will not be collected and it will be discarded. |
| **Who will make initial contact with participants?** | The Surgeons, Fellows and Registrars will have initial contact with the patients. These patients will have been referred to the Surgeon’s rooms or the Hospital’s Breast Clinic by GPs or specialists or from the BreastScreen NSW Program for consideration for surgery as part of routine standard-of-care. |
| **Who will perform the consent process? How will this be carried out?** | Consent for participation in the trial will take place during the preadmission clinic visit. This will be carried out by a medical officer who is a researcher and independent from the attending medical team. |
| **Will participants be consented verbally/explicitly/using eConsent?**  [SLHD Research Forms Link](https://www.slhd.nsw.gov.au/rpa/Research/forms.html) | Written consent will be obtained. |
| **Will participants be given a specific time period to consider participating?** | Yes. They will be given at least 1 week consider participating. |
| **Review of existing databases or databanks (please identify the database/databank and the custodian)** | Yes. The electronic records database of Chris O’Brien Lifehouse will be accessed for histopathology results of lymph node biopsies. |
| **Review of clinic files (please include who will be reviewing these files, for example a research coordinator).** | Not applicable. |
| **Advertisements (please include where the advertisement will be placed for example, in a newspaper, poster in a clinic or hospital foyer, radio announcements, website etc.)** | Not applicable. |
| **Information Letter to Medical practitioners** | No |
| **Explain how potential participants will be screened for the study** | The Surgeons, Fellows and Registrars will have initial contact with the patients who have been referred to the Surgeon’s rooms or the Hospital’s Breast Clinic by GPs or specialists or from the BreastScreen NSW Program. This is all part of routine standard-of-care. In addition to routine care. A medical officer who is a researcher and independent from the attending medical team will assess the patient for inclusion and exclusion criteria for the trial. They will be consented for participation at a later stage after at least 1 week is given for them to consider participation. |
| **Any other potential recruitment methods.** | No |

### 6.5. PARTICIPANT ENROLMENT

Potential participants will be enrolled into the study after the informed consent process has been completed for their routine breast cancer reconstruction surgery. The participant will be assessed to see if they meet all the inclusion and exclusion criteria. Study participants that do meet the criteria will receive a study enrolment number and this will be documented in the participant’s record on the study’s database.

### 6.6. INFORMATION AND CONSENT

The patient will be consented for their procedure as usual. Additional written informed consent will be requested for their participation in this trial. They will receive a Participant Information Sheet.

### 6.7. RANDOMISATION PROCEDURE

None.

6.8. END OF STUDY TREATMENT/WITHDRAWAL PROCEDURE

A patient may withdraw from having their data used for the study at any time. This will not affect their ongoing care postoperatively. Withdrawn participants will be replaced in order to attain the required number of participants to maintain enough statistical power.

### 6.8. PATIENT WITHDRAWAL

A participant may withdraw from the study for any reason. They may notify the Investigator, Chu Nguyen, by phone or by email (details provided on Patient Participant Information Sheet). If this happens, their data will be erased from the study’s database. They will continue to have routine care pre-, intra- and post-operatively.

# 7. OUTCOMES

### 7.1. DEFINITION OF OUTCOMES

PRIMARY OUTCOMES

1. Number of sentinel lymph nodes identified with ICG and 99mTc methods

* Number detected by 99mTc alone, ICG alone and by both techniques.

1. Metastatic nodes positive and negative for 99mTc and ICG

* Number of SLNs with cancer detected by 99mTc alone, ICG alone and by both techniques.

SECONDARY OUTCOMES

1. The surgeon’s reported ease of identifying SLNs with ICG compared to 99mTc.

* Assessed with a survey using a Likert scale that includes very easy, easy, neutral, difficult and very difficult.

1. Any complications related to ICG and 99mTc axillary sentinel lymph node biopsy.

Postoperative complications include:

* Infection (Time Frame: 30 days postoperative)

Superficial, Deep or Organ space surgical site infection as defined by the Centres for Disease Control and Prevention (CDC). Patients treated with oral or IV antibiotics will be documented, including patients who have loss of implant due to infection.

* Seroma/Haematoma (Time Frame: 30 days postoperative)

Any evidence of seroma/ haematoma will be documented based on requirement of a surgical intervention or non-surgical management.

* Wound Dehiscence (Time Frame: 30 days postoperative)
* Readmission or other complications (Time Frame: 30 days postoperative)

# 8. INVESTIGATIONAL MEDICINAL PRODUCT

### 8.1. DESCRIPTION OF INVESTIGATIONAL PRODUCT

The drug is Indocyanine green and the imaging device is the SPY PHI System.

a. Generic name: Indocyanine green dye/ SPY Elite System

b. Brand: Infracyanine® (SERB, Paris, France) / Novadaq Technologies Inc. (Mississauga, Canada)

c. Strength: 25mg/10mL

d. Form: Injection

e. Route: IV

f. Supply: The indocyanine green dye is sourced by Chris O’Brien Lifehouse Pharmacy

g. PBS and ARTG listing status (for Australian studies): ARTG Special Access Scheme Category C (M58); Not PBS listed

h. Standard of Care: Standard of care management of sentinel lymph node biopsy at our institution currently involves use of 99mTc only.

### 8.2. PHARMACOKINETICS

Indocyanine green is a fluorophore, tricarbocyanine iodide dye and has a short plasma half-life of three to four minutes.18, 19 When indocyanine green is administered intravenously it is rapidly and extensively bound to plasma protein and when exposed to near-infrared light it emits fluorescence. The fluorescence imaging system that will be used in this study is the SPY-PHI (Novadaq, Mississauga, Canada). Fluorescence images are recorded in real-time with a digital camera which allows for lymphatic mapping. And has a short plasma half-life of three to four minutes.19

### 8.3. DOSE (INCLUDING DISPENSE SCHEDULE)

A 0.5mL dose of Infracyanine® 25mg/10mL is given stat subdermally in the peri-areolar area after anaesthesia and just prior to the surgery commencing.

### 8.4 ADMINISTRATION

1. Concomitant medications, including medications required for the management of AE’s:

Methylprednisolone and anti-histamines may be used if there is an anaphylactoid reaction.

2. Prohibited Medications: Not applicable.

3. Dose modifications: The dose is not weight dependent but will be a stat dose of 0.5mL

4. Missed doses/patient compliance: Not applicable as this is a stat dose intraoperatively.

### 8.5 HANDLING AND STORAGE OF STUDY DRUGS

The vial is stored away from light and humidity and below 25 degrees Celsius.

### 8.6 FUNDING

The indocyanine green has already been approved by the Chris O’Brien Lifehouse Drug and Therapeutics Committee under the TGA Special Access Scheme.

**9. DATA COLLECTION**

9.1. PARTICIPANT REGISTRATION

The REDCap database (The University of Sydney) will allow for storage of identifiable patient data which will auto generate a de-identified record within a separate research data project in the REDCap system using a “Record ID” as a participant identifier.

9.2. FORMS AND PROCEDURE FOR COLLECTING DATA

All data will be collected using the REDCap database created. This proforma will contain all variables required including patient demographics, intra-operative information and outcomes at followup visits postoperatively.

9.3. CASE REPORT FORMS AND SCHEDULE FOR COMPLETION

Case report forms will be created using the REDCap database. These will be stored in a locked cabinet in the Breast Theatre at Chris O’Brien Lifehouse.

# 10. QUALITY CONTROL AND ASSURANCE

### 10.1. CONTROL OF DATA CONSISTENCY

### 10.2. AUDITS

Audits will be discussed between the Investigators and Chief Investigators and held at regular intervals throughout the trial period.

### 10.3. PROTOCOL AMENDMENTS

Protocol amendments will be discussed between the Investigators and Chief Investigators before being drafted for submission to the Ethics Committee.

# 11. ETHICS

### 11.1. INVESTIGATOR AUTHORISATION PROCEDURE

The conduct of this study will commence once the initial approval process has been completed through Ethics and Governance authorisation for each study site. This includes approved versions of the participant information and consent form.

Updated documents will only be implemented once they have been reviewed and approved by an Ethics Committee and if applicable Governance Officer for each site.

### 11.2. PATIENT PROTECTION

The responsible investigator will ensure that the study is completed in accordance with the guidelines set out in the [National Statement on Ethical Conduct in Human Research](http://www.nhmrc.gov.au/guidelines/publications/e72) (2007) (the National Statement) and the [CPMP/ICH Note for Guidance on Good Clinical Practice](http://www.tga.gov.au/industry/clinical-trials-note-ich13595.htm) and any other relevant legislation/guidelines.

# 12. SAFETY

In Australia, indocyanine green is a Special Access Scheme Category C drug that can be approved by the TGA for intraoperative diagnostic use. It has an excellent safety profile with a low incidence of adverse events.20, 21

The NSW Health Policy on Safety Monitoring and Reporting for Clinical Trials (PD2017\_039) and the NHMRC Guidance: Safety Monitoring and Reporting in Clinical Trials Involving Therapeutic Goods 2016 (the NHMRC Guideline) have been referred to for this section of the study protocol.

This trial will be conducted under the Clinical Trial Notification (CTN) Scheme. The TGA may conduct an audit of the trial and stop the trial in the public’s interest if necessary.

### 12.1. ADVERSE EVENT REPORTING

Detailed records of all reported adverse events will be kept and maintained so that they are up-to-date.

There will be an annual safety report which will include:

* Analysis of new and relevant findings
* Analysis of the safety profile of indocyanine green
* Discussion of the implications of the safety data in relation to the trial’s risk-benefit ratio
* Discussion of any measures taken or proposed to reduce the risk of adverse events.
  1. All adverse events will be managed medically. Once the patient is stable, the event will also be reported through the Incident Information Management System (IIMS) as per hospital protocol and escalated to the Chief Investigator.

### 12.2. SERIOUS ADVERSE EVENT REPORTING

All serious adverse reactions occurring will be reported to the TG. Fatal or life threatening “suspected unexpected serious adverse reactions” (SUSARs) will be managed medically immediately. These SUSARs will then be reported to the TGA immediately and no later than 7 calendar days after being made aware of the case. Followup information will be reported within a further 8 calendar days. All other SUSARs will be reported to the TGA no later than 15 calendar days after being made aware of the issue.

The TGA, HREC and investigators will be notified of all significant safety issues that negatively affect the safety of participants or negatively affect the continued ethical conduct of the trial. Significant safety issues that meet the criteria for an urgent safety measure will be notified within 72 hours. All other significant safety issues will be notified within 15 calendar days after being made aware of the issue. These include lack of efficacy of indocyanine green, a major safety finding from another study related to this trial and recommendations from the Data Safety Monitoring Board.

The site investigators will assess all local safety events and provide appropriate medical care as indicated. A report will be given to the sponsor containing all relevant information so that an appropriate safety analysis can be performed. The investigator will report to the sponsor within 24 hours of becoming aware of all serious adverse events. The investigator will report within 72 hours of becoming aware of the event of all significant safety issues.

The institutions are also the trial sponsors. It will be the sponsor’s responsibility to oversee the safety information in the trial. The sponsor will assess whether any safety issues have medico-legal risk, affect the responsible conduct of research or affect the trial’s continued site authorisation. The sponsor will carry out corrective and preventative action.

### 12.3. DATA SAFETY AND MONITORING BOARD (DSMB)

A Data and Safety Monitoring Committee (DSMC) will be established to oversee the safety aspects of this trial. The DSMC will consist of a statistician and two surgeons with expertise in breast surgery. They will be independent of any of the attending medical team and research team. The DSMC members will not have any vested interest in the outcome of the trial. Ad-Hoc members will be appointed if further specific expertise is needed. The role of the DSMC is to provide independent oversight and ensure that the trial is conducted safely and ethically. It is anticipated that the DSMC meets six-monthly. The DSMC will review and evaluate adverse events and outcome measures. The results of the analyses by the DSMC will not be shared with the site investigators unless specific action is required. The DSMC will develop rules for stopping the trial. The members of the DSMC will shared with SLHD Research Ethics & Governance Office once they have been appointed.

### 12.4. EARLY TERMINATION

If this study is decided to be terminated, the Investigator, Chu Nguyen, will be responsible for informing participants, correspondence to HREC, and compiling a final study report.

# 13. BLINDING AND UNBLINDING

Not applicable.

# 14. CONFIDENTIALITY AND STORAGE AND ARCHIVING OF STUDY

The Management of Data and Information in Research: A guide supporting the Australian Code for the Responsible Conduct of Research has been referred to. The Australian Code for the Responsible Conduct of Research (2007) has been referred to.

Submission of a Research Data Management Plan (RDMP) is included in this submission. Data will not be stored in Excel or Word.

All data will be stored in the REDCap database (The University of Sydney) and in the Case report forms which will be stored in a locked cabinet in the Breast Theatre at Chris O’Brien Lifehouse.

The database will allow for storage of identifiable patient data which will auto generate a de-identified record within a separate research data project in the REDCap system using a “Record ID” as a participant identifier. Confidentiality will be maintained throughout the study and for archiving and storage as only the principal investigator will have access to the database.

There will be plans to use this data in future for a cost-effectiveness analysis. If there is a need to share the data in future, this will be discussed with between the Chief investigators. The data will be retained for 15 years post data analysis.

Data collection is the responsibility of the research staff of Chris O’Brien Lifehouse under the supervision of the investigator, Chu Nguyen. This investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported.

The investigator, Chu Nguyen, will be interpretating the data. This will be presented to the Chief Investigators Sanjay Warrier and Carlo Pulitano for analysis, review of tables and listings and plans for reporting.

# 15. TRIAL SPONSORSHIP AND FINANCING

All the costs related to the study will be supported by Chris O’Brien Lifehouse.

# 16. INDEMNITY

16.1. COMPENSATION

The main harm is from an adverse reaction to the intravenous injection of ICG. The patient’s anaphylactoid reaction will be managed immediately by the medical team. Risk will be mitigated during the participant screening as previous allergies will be identified and the patient excluded from the study if indicated.

In the event of a harm occurring, the compensation arrangements will follow the [Medicines Australia Guidelines for Compensation for Injury Resulting from Participation a Company-Sponsored Clinical Trial.](http://medicinesaustralia.com.au/issues-information/clinical-trials/indemity-and-compensation-guidelines/)

**17. PARTNERING WITH CONSUMERS**

The Consumer Involvement and Engagement Toolkit (the Toolkit) from the Australian Clinical Trials Alliance (ACTA) and Health Consumers NSW will be utilised to guide this process.

The investigators will incorporate input from consumers in the delivery of the trial. This requirement will be met over the net 18 months. The first step in this plan will occur over the first 3 months. Consumers, community members and potentially organisations will be sought to contribute their consumer perspective to the trial. Once a group has been formed, meetings will be arranged so that discussions can be had regarding the anticipated benefit of the research, ideas for activities that consumers can be involved in, expectations of all members, and any support and training that is needed. Given that the research questions already have been decided the input sought from consumers and community members will be mainly related to their involvement in the delivery of the trial and potential future studies related to the research topic to address any gaps in knowledge.

The subsequent meetings will be planned with consumers and community members as results of the trial are analysed at significant timepoints such as 6 months, 12 months and 18 months. Consumers and community members will be invited to have discussions about the research findings as they offer a different perspective than the medical and research team. As the trial nears the end and data is being finalised, consumers and community members will be invited to help develop plain language summaries of research results and findings. The ultimate goal of this research is to develop a new protocol for axillary sentinel lymph node biopsy using ICG if it is evaluated to be safe and effective. Consumers and community members will be invited to discussions regarding the plan to translate the final trial results into policy and practice guidelines.

Consumers and community members will also be invited to discuss methods of disseminating the results of the trial. This could include presentations at consumer and community events, and publications in consumer and community media.

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