Intraoperative evaluation of axillary lymphatics for breast cancer patients undergoing axillary surgery

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1. Project Summary

Lymphedema is an incurable and chronic condition where damage or blockage to the lymphatic system leads to swelling, pain, numbness, recurring infections, and fibrosis.[1-3] Women diagnosed with breast cancer undergoing axillary lymph node surgery may develop lymphedema due to damage or blockage of the lymphatic vessels that drain the arm. Rates range from 6-46% depending on the extent of axillary surgery.[1-3]

Axillary reverse mapping (ARM) is a technique performed by breast surgical oncologists as a way to potentially decrease the risk of lymphedema from axillary surgeries.[4-14] ARM identifies the lymphatic channels draining from the arm during primary breast tumor resection and axillary lymph node surgery, thereby differentiating those draining the breast vs those draining the arm. To date, ARM is most commonly performed using either a blue dye[4-7,10] or immunofluorescence[9-15]. At the time of axillary node surgery, the dye is injected in the subcutaneous fat in the upper arm, resulting in enhanced visualization of lymphatics. These lymphatics may then be spared, reducing the risk of lymphedema. However, significant limitations to the use of blue dye in lymphatic mapping exist. To visualize blue dye, the surgeon must dissect through the soft tissue directly to a blue lymphatic, which can result in variable success in identifying the ARM nodes and/or lymphatics, as well as a persistent blue stain at the site of injection.[9,11,12]

Compared to blue dye, fluorescence-guided surgery (FGS) with indocyanine green (ICG) dye represents an opportunity to greatly enhance the potential of the ARM procedure to increase the surgeon's confidence in identifying the lymphatic architecture before focusing on the operative field. FGS allows the surgeon to visualize lymphatics without the need to dissect directly to the lymphatic channels; rather than directly exposing the lymphatics with surgical dissection, the lymphatics can be viewed on the imaging platform. In one prospective study evaluating the ARM procedure, patients underwent injection with both isosulfan blue dye and ICG. This study reported that blue dye and ICG identified a similar number of lymph nodes but ICG identified greater number of lymphatics draining the arm.[10] However, challenges also exist in the use of FGS with ICG for ARM surgery. Commercially available imaging platforms to support fluorescence-guided surgery do not work with ambient lighting. Ambient room light contamination of near-infrared fluorescence (NIRF) signal emitted from fluorescence dye injected into the patient typically requires ambient lights in the operating room to be dimmed or turned off to capture the fluorescence images. This limits surgeons' ability to use FGS in real-time. Some FGS imagers attempt to compensate for ambient light through background subtraction, which leads to reduced image quality due to dynamic range compression, reduced sensitivity due to shorter effective collection times, and added display latency.[16]

In this proposal, we will assess the feasibility of using the Asimov Imaging Platform for fluorescence-guided surgery along with ICG in the operating room for the ARM procedure. FGS with ICG is an acceptable approach to performing the ARM procedure nationally and internationally[9-15]. Theoretical benefits to the Asimov Imaging Platform exist, specifically that it can be used without altering external (i.e., room) light functionality or levels, which will enable real-time visualization of lymphatics using both fluorescence and familiar anatomical reference points to support ARM.

The objective of this study is to assess the feasibility of using fluorescence-guided surgery with ICG dye through the Asimov Imaging Platform for the axillary reverse mapping procedure. We will use blue dye to guide clinical decision making. We will compare the ICG fluorescence findings (research) with the blue dye (standard of care). Nationally and internationally, both blue dye and FGS with ICG dye are used[4-14]. In this study, we are specifically evaluating the feasibility of the Asimov Imaging Platform for fluorescence-guided surgery with ICG dye.

2. Background and Significance

Breast cancer is the most common cancer among women globally, with more than 2 million new cases diagnosed annually. In the United States, more than 250,000 women a year are diagnosed with breast cancer. Surgery is central to cancer treatment, with more than 90% of these patients undergoing surgery for tumor resection, mastectomy, or breast reconstruction.[17] Survival of breast cancer patients has increased substantially as a result of improved, multidisciplinary treatment approaches which means that survivors are also living longer with any morbidity resulting from treatment, including lymphedema.

Lymphedema is an incurable and chronic condition where damage or blockage to the lymphatic system leads to swelling, pain, numbness, heaviness, recurring infections, and fibrosis.[18] Women undergoing axillary lymph node surgery may develop lymphedema due to damage or blockage of the lymphatic vessels that drain the arm. In affected breast cancer patients, lymphedema often manifests in the arm weeks to years after the removal of the primary tumor or radiation therapy. Rates of lymphedema range from 6%-46%, depending on the extent of axillary surgery.[1-3] A recent systematic review of 72 clinical studies (N=29,612 women) revealed that approximately 1 in 5 women who survive breast cancer will develop arm lymphedema.[3] Not only does lymphedema reduce quality of life, it has long been a feared complication of surgical cancer treatment impacting survivorship: fear stems from a patient's concerns regarding the chronic, progressive nature of the condition and the clinician's relative inability to predict or prevent it from occuring.[19] Further, associated per patient costs range from $3000 to $16,000 annually.[18]

Because lymphedema may result from iatrogenic damage to upper limb lymphatics during axillary lymph node surgery, identification and preservation of arm lymphatics decreases the likelihood of lymphatic disruption during axillary lymph node surgery, leading to a lower incidence of upper limb lymphedema.[4,5,8] Axillary surgery has historically consisted of an axillary lymph node dissection (ALND) where between 15 to 20 lymph nodes were removed from the axilla. Lymphedema rates for an ALND range between 20% and 40% in published studies.[1-3,19] Because of the associated morbidity rates, this procedure is now only performed for patients with confirmed axillary disease. For women with clinically negative axillary lymph nodes, axillary staging is performed with a sentinel lymph node (SLN) biopsy. The SLN biopsy procedure involves pre-operative mapping of the lymphatics to identify one or two lymph nodes where the breast would drain to first if the disease had spread. These lymph nodes are then removed through a small incision. Lymphedema rates are lower with this procedure, but still range from 5% to 7% in prospective randomized controlled trials.[20]

Axillary reverse mapping (ARM) is a technique performed by breast surgical oncologists as a way to potentially improve the quality of axillary surgeries.[4-14] As shown in **Figure 1**, ARM is utilized to identify the lymphatic channels from the arm and differentiate them from the breast lymphatics during primary tumor resection and axillary lymph node surgery. To date, ARM is most commonly performed using a blue dye or immunofluorescence, which is injected in the subcutaneous fat in the upper arm, resulting in enhanced visualization of lymphatics at the time of axillary node surgery.[5] Studies have shown that lymphedema rates decrease three-fold when using ARM.[4-6,8]

Significant limitations to the use of blue dye in lymphatic mapping exist, including its low optical depth penetration, insufficient identification rates of the ARM nodes and/or lymphatics, as well as a persistent blue stain at the site of injection. Most importantly, the blue lymphatics can only be identified with direct dissection onto the lymphatic, something that is challenging when using a small incision (as for a SLN biopsy) or in obese patients (where the blue lymphatics are deeper and surrounded by adipose tissue making them more difficult to identify). Finally, the blue lymphatics from the arm may actually drain outside the operative field and never be visualized due to its low visual penetration depth; in these situations, the breast surgeon is less confident whether the blue lymphatic was inadvertently divided, or whether it was tracking along a different route.

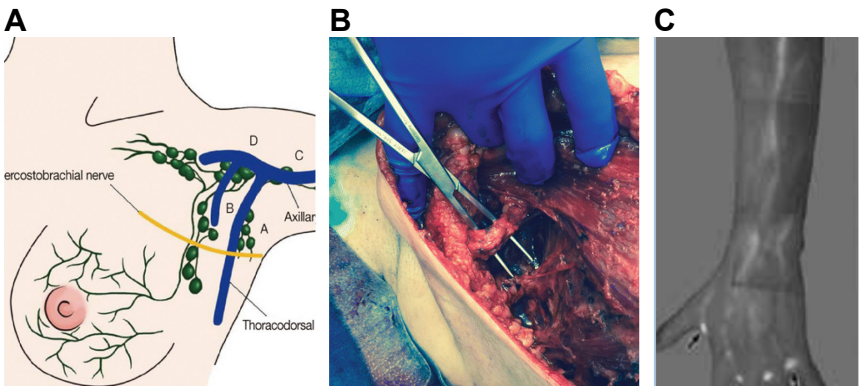

Figure 1. Axillary reverse mapping (ARM). (A) Sentinel lymph nodes and typical lymphatic drainage patterns from the breast are identified (green).[21] Lymphography with (B) blue dye is challenging due to the non-specific binding to the lymphatics (blue) and poor signal retention.[8] (C) Lymphatic vessels are shown clearly with ICG lymphography (white).[22] Although ICG easily highlights large lymphatic vessels, small and critical lymphatic vessels, which can be as small as 1 mm in diameter, are unable to be seen. *The clinical need in spatial and sensitivity resolution highlights the need for improved FGS imaging systems.*

Fluorescence-guided surgery (FGS) with indocyanine green (ICG) represents an opportunity to greatly enhance the potential of the ARM procedure to increase the surgeon's confidence in identifying the lymphatic architecture before focusing on the operative field.[16] As ICG has shown to offer deeper optical penetration than blue dye with ability to detect lymphatic channels located up to 2 cm deep, surgeons can follow the route of drainage from the injection site through lymphatic vessels to preserve its patency.

In this proposal, we will use the Asimov Imaging Platform for fluorescence-guided surgery in the operating room. The Asimov can be used without altering external (i.e., room) light functionality or levels. This will enable real-time visualization of lymphatics to support ARM and would represent a significance advancement.

Technical gaps in the literature limit our understanding of ICG fluorescence for ARM that impact implementation of this approach within the clinical workflow. Specifically, we need to quantify the critical image acquisition parameters that provide 1) surgeons' detection contrast (CNR) of post-injection time and 2) fluorescence signal retention of lymphatic vessels. The post-injection time is valuable for elucidating the optimal clinical workflow. Although some studies inject more than two hours before surgery, others inject immediately before the axillary surgery.[9] In healthy patients, ICG typically reaches the nearest draining lymph node within 15 minutes,[23] but because lymphatic clearance and backflow are dependent on the damage to vasculature, the optimal post-injection imaging time point will vary depending on the severity of damage to lymphatic vasculature. Thus, the clinical workflow is difficult to establish and optimize.

The fluorescence signal retention is necessary to enable surgeons to identify and be able to see these lymphatic vessels during surgery. Previous studies have not provided these parameters, largely due to inherent limitations in quantification capabilities of existing fluorescence imaging technology. Furthermore, existing fluorescence imaging technology does not provide fluorescence overlay on the white light reflectance image. This visualization is critical to provide surgeons with familiar anatomical reference points seen with white light that are further enhanced with fluorescence. This fluorescence illuminates the lymphatic flow and its location to support appropriate navigation around critical structures to preserve patency when possible.

3. Study Objective and Specific Aims

Specific Aim 1: To determine the feasibility of using ICG fluorescence through the Asimov Imaging Platform for axillary reverse mapping by comparing lymphatic visualization using blue dye (standard of care) versus ICG fluorescence imaging.

Specific Aim 2: To define optimal image acquisition parameters of using ICG fluorescence through the Asimov Imaging Platform for axillary reverse mapping to inform the clinical workflow.

2a. To define dynamic range of ICG fluorescence signal in lymphatic vessels and background.

2b. To assess image contrast as a function of ICG dose and time post-injection.

4. Research Design and Methods

4.1. Study Overview

Lymphatic drainage from the upper arm is often different from that of the breast, allowing safe removal of only the lymphatics of the breast and protection of the lymphatic channels draining the upper extremity during axillary dissection (AD) or sentinel lymph node (SLN) biopsy, thereby reducing the risk of arm lymphedema.[4-14] In this prospective study, breast cancer patients undergoing SLN biopsy (n=0-20) or axillary lymph node dissection (n=0-15) will be enrolled to undergo axillary reverse mapping (ARM) using isosulfan blue dye. Patients will also receive ICG injection with visualization through the Asimov Imaging Platform to allow comparison of blue dye versus ICG lymphatic identification.

We will also define optimal image acquisition parameters of using ICG fluorescence through the Asimov Imaging Platform for ARM. We will use quantitative fluorescence to define optimal image acquisition parameters. In order to elucidate injection technique superiority, the critical image acquisition parameters that provide surgeons detectable contrast (CNR) of post-injection time, and fluorescence signal retention of lymphatic vessels will be measured. The post-injection time is valuable for elucidating the optimal clinical workflow. The fluorescence signal retention is necessary to enable surgeons to identify and be able to see these lymphatic vessels during surgery.

4.2. Selection of Participants

Inclusion criteria:

- ≥ 18 years of age
- Diagnosis of breast cancer requiring surgical lymph node evaluation either by sentinel lymph node biopsy or axillary lymph node dissection
- Surgery at University of Wisconsin Hospital and Clinic

Exclusion criteria:

- Pregnant or breast feeding
- Unable to provide informed consent
- Allergy to indocyanine green
- Patients with clinically positive lymph nodes undergoing sentinel lymph node biopsy, with or without axillary lymph node dissection, after neoadjuvant chemotherapy

4.3 Screening and recruitment:

Patients being cared for by Dr. Neuman within the UW Breast Center will be eligible for consideration. Dr. Neuman will identify eligible patients from her clinical practice and introduce the study to them at the time of their clinic visit. A clinical research coordinator will then discuss the study in detail and obtain informed consent. Participants will have sufficient time to consider participating in this study.

4.4. Consent Process

The consent process will occur at the Breast Center. Subjects will be consented in a closed private room by a member of the study team. As much time as needed will be given for potential subjects to decide whether or not to participate in this study. Subjects will receive a copy of the consent form and

will be given contact information for study coordinator and PI for any additional questions. Subjects will be informed that they are not obligated to participate in the study.

4.5. Study procedures:

Standard of Care Procedures:

Axillary surgery: Axillary lymph node dissection or sentinel lymph node biopsy will be performed as indicated by the patient's clinical history. For patients enrolled in the study, sentinel lymph node mapping and biopsy will be performed using technetium-99m sulfur colloid only (no injection of isosulfan blue dye in the breast), which is an acceptable standard of care. The participating surgeon will otherwise follow their usual practice for performing these routine procedures.

Axillary reverse mapping: Axillary reverse mapping is an acceptable standard of care and has been used internationally (including at the UWHC) to reduce the risk of lymphedema for women undergoing axillary surgery.[4-14] ARM is also currently being studied in an NCI funded trial to determine the extent to which ARM reduces lymphedema risk (NCT039270127). We will use the ARM procedure for both the axillary lymph node dissection and the sentinel lymph node biopsy. Although the risk of lymphedema is lower after a sentinel lymph node biopsy than with an axillary lymph node dissection, it can still occur (estimated 5-7%).

To perform ARM, 2.5 ccs of 1% isosulfan blue dye (10 g/L concentration) will be injected subcutaneously in the volar surface of the ipsilateral upper extremity at the bicipital sulcus after induction of anesthesia. Participating surgeons at UW Health have experience performing ARM with isosulfan blue dye. During the axillary surgery, identification of lymphatics will be attempted. If lymphatics are visualized in the surgical field, lymphatics draining the arm that do not also have crossover breast drainage will be spared or reapproximated. Importantly, the ARM procedure does not influence the extent of an axillary surgery, as it does not determine whether or not lymph nodes are involved with cancer. Rather, the dye maps the pathway of arm lymphatic flow so that these lymphatics can potentially be spared during an axillary surgery. The breast surgeon will use the blue dye to guide clinical decision-making for the ARM procedure.

Research Procedures:

ARM mapping with ICG fluorescence using the Asimov Platform: The research procedures will include using ICG fluorescence for the ARM mapping. OnLume will provide a sterile drape designed specifically for the OnLume Asimov. 25 mg of ICG and 10 mg Sterile Water will be dispensed for injection by UW Health pharmacy. Before the image acquisition of each patient, ICG will be reconstituted under sterile conditions with Sterile Water for Injection(2.5 mg/1 ml). Shake the vial gently to dissolve. Reconstituted ICG must be used within 6 hours after reconstitution. The total dose of dye will be kept below 2 mg/kg of patient body weight.

ICG dosing described in the literature has varied significantly (Table 1). A total dose of 1 ml (2.5 mg) of ICG will be injected subdermally, in divided doses. 0.5 ml will be injected in the subcutaneous fat in the volar surface of the upper arm at the bicipital sulcus and 0.5 ml in the 2nd interdigital space. The two injection protocol follows the published experience by Abacci, et al. with the goal to improve ICG's circulation through the lymphatic vessels.[24] The upper arm injection will be performed within 1 cm of the isosulfan blue dye injection and will occur after induction of anesthesia. At least two minutes of massage will be performed to promote upper limb lymph flow. Visualization of the lymphatics using the Asimov Platform will be attempted

Table 1. Summary of Injections Methods for ICG in ARM Procedure		
Author	**Dose of ICG injected**	**Injection Location**
Noguchi[12]	0.1 ml (0.25 mg)	Inner wrist
Noguchi[11]	0.1 ml (0.25 mg)	Inner wrist
Ikeda[25]	0.5-1ml (5 mg)	Upper inner arm
Ikdea[26]	0.5-1 ml (5 mg)	Upper inner arm
Sakurai[13]	0.15 ml	Interdigital area
Foster[10]	0.5-3 mg/ml	Upper arm
Ma[15]	1 ml (0.5 mg/ml)	Upper arm
Yuan[14]	1 ml (2.5 mg)	Upper arm
Abacci[24]	1 ml (2.5 mg/ml) in divided doses	Upper arm, 2nd interdigital space

Subjects will receive fluorescence imaging using the Asimov Imaging Platform. Beginning at the time of ICG injection, image and video capture of white light reflectance and fluorescence will be collected.

Impact on clinical care of the ICG mapping: The objective of this study is to evaluate the feasibility of using ICG fluorescence *through the Asimov Imaging Platform*. Compared to other platforms, the Asimov Imaging Platform allows real time imaging without altering ambient light. The presence of ICG fluorescence visualized lymphatics will be documented per the research protocol, but will not be used to influence surgical decision making.

4.5. Data Collection

Operative data related to ARM: For each case, we will also assess whether blue dye identified lymphatics were visualized (yes/no) in the nodal basin and whether the blue lymphatic was spared (yes/no). We will also assess whether ICG identified lymphatics were visualized (yes/no) in the nodal basin and whether the ICG lymphatics were spared (yes/no). Surgical decision making will be guided by the blue dye only and the ICG fluorescence will not be considered. However, we will still document whether or not the ICG fluorescent lymphatics were spared. We will assess whether intact lymphatic channels were present at the completion of the axillary surgery via both techniques (yes/no/not assessable). We will record time at start of case, any fluorescent imaging, and completion of axillary surgery (defined as removal of last lymph node).

Chart review demographics and tumor characteristics: We will record other limited demographics about the patient including: Age, race/ethnicity, BMI, type of axillary surgery, type of breast surgery, receipt of neoadjuvant systemic therapy, prior axillary surgery, prior receipt of radiation, clinical and pathologic cancer stage (tumor size and lymph node status), and receptor status (estrogen receptor, progesterone receptor, her2neu). We will record the number of lymph nodes removed. We will indicate whether removed lymph nodes had radiotracer, blue dye, or fluorescence. For patients undergoing SLN biopsy, this will allow us to assess whether the SLN is the same as the ARM identified node. We will evaluate for any additional axillary surgeries or complications related to axillary surgery (infection, lymphedema) until 1 year post-surgery. We will document receipt of post-operative radiation.

Technical data related to ICG fluorescence imaging: Beginning at the time of ICG injection, image and video capture of white light reflectance and fluorescence will be collected. Following the operation, post-processing will be conducted to elucidate injection technique superiority. Physicians will confirm software-assisted lymphatic labels in the video and measure vessel diameters.

We will use quantitative fluorescence to define optimal image acquisition parameters. In order to elucidate injection technique superiority, the critical image acquisition parameters that provide surgeons detectable contrast (CNR) of post-injection time, and fluorescence signal retention of lymphatic vessels will be measured.

4.6. Analysis Plan:

1. Aim 1: To determine the feasibility of using ICG fluorescence for axillary reverse mapping through the Asimov Imaging Platform by comparing lymphatic visualization using blue dye (standard of care) versus ICG fluorescence imaging: Given the increased optical penetration with the ICG lymphatics, we anticipate greater visualization with the ICG. The proportion of cases where blue dye visualized lymphatics can be seen after 5 minutes post-injection will be reported along with the corresponding 95% confidence interval, which will be constructed using the Wilson score method. The same proportion will be reported for the ICG lymphatics. Comparisons between visualization of lymphatics by blue dye versus ICG fluorescence imaging will be made using test of two proportions. We will also compare whether lymphatics were able to be spared and whether intact lymphatic channels were able to be confirmed via both techniques. We will also generate descriptive statistics of patient characteristics associated with visualization of lymphatics with each approach to explore clinical scenarios when an approach may be less effective. For comparisons, the blue dye is considered the standard of care comparison.

2. Aim 2: To define optimal image acquisition parameters of using ICG fluorescence through the Asimov Imaging Platform for axillary reverse mapping.

   Aim 2a. To define dynamic range of ICG fluorescence signal in lymphatic vessels and background: Imaging acquisition parameters generated from image snapshots and videos will be summarized using standard descriptive statistics, including means, standard deviations, medians and interquartile ranges to characterize the dynamic range of ICG fluorescence signal in lymphatic vessels and the background. Nonlinear regression analyses will be conducted to statistically model CNR as a function of ICG dose and time.

   Aim 2b. To assess image contrast as a function of ICG dose and time post injection: Image contrast (CNR) will be measured for these lymphatics vessels and a time-curve will be developed that demonstrate the change in contrast over time. The magnitude of fluorescence signal retention of ICG in the lymphatic vessels, which is a function of both pharmacokinetics/pharmacodynamics of ICG and the sensitivity of the imaging platform, will be measured. Detectable contrast is defined by the Rose criterion: CNR>3.

4.7. Sample Size:

We will plan to enroll approximately 0-20 patients undergoing SLN biopsy and 0-15 patients undergoing an ALND for a total of about 25 patients. The proposed total sample size is sufficient to generate descriptive statistics with an adequate level of precision.[16] Specifically, the proportion of cases where lymphatics can be seen after 5 minutes post-injection will be estimated with a standard error of less than 12% and the corresponding 95% confidence interval will be no wider than 40%.

No power calculation is calculated for this feasibility study.

4.8. Risks and Benefits to the Participant

Potential risks: Potential risks from ICG are minimal as it is a well-known, FDA-approved dye that has a very good safety profile (adverse event rate: 1 in 42,000) when administered intravenously.[9,16,27] Adverse events can range from a redness of the skin or hives, up to cough or difficulty swallowing. In the operating room, these would be treated with medication such as antihistamines, H2 blockers,

and/or epinephrine. Although ICG is approved for intravenous administration, it has been safely injected into interstitial, subcutaneous (SC) or intradermal locations in hundreds of patients, including breast tissue. A summary of ICG risks by publication on ARM Procedures are shown below in Table 2, indicating the low risk profile:

Table 2. Summary of ICG risks for ICG in ARM Procedure		
Author	**Patient population**	**Number of adverse reactions/allergies**
Noguchi[12]	*Review article*	
Noguchi[11]	131 patients	None reported
Ikeda[25]	98 patients	Zero.
Ikeda[26]	60 patients	None reported
Sakurai[13]	372 patients	Zero.
Foster[10]	23 patients	Zero.
Ma[15]	44 patients	None reported
Yuan[14]	689 patients	None reported

Potential risks from the use of the OnLume Asimov Imaging platform are low since the imaging device will be used in a similar manner as its FDA 510(k) clearance. The duration of the surgery is not expected to be significantly longer in duration (<20 minutes) with the additional use of the OnLume System imaging than with just the standard of care. Per discussions with anesthesia staff, the additional time for anesthesia poses no foreseeable additional risk to the patient. The risk associated with the injection of the dye will be minimized by close observation of the patient after injection. Injection will occur in the OR when extensive monitoring is already occurring.

As with any study, there is a remote risk of breach of confidentiality. This risk has been minimized through data protection efforts. Only approved study personnel will have access to the direct identifiers.

Patients enrolled in this study who are undergoing the sentinel lymph node biopsy will have lymphatic mapping of the breast performed using technetium-99m sulfur colloid only (no injection of isosulfan blue dye in the breast). The sentinel lymph node biopsy was initially described with blue dye only, but quickly incorporated the technetium-99m sulfur colloid as it increased the ease of use and decreased mapping failure rates. In current clinical practice, surgeons may choose to perform lymphatic mapping with blue dye only, technetium-99m sulfur colloid only, or both. All methods are considered an acceptable standard of care. Use of technetium-99m sulfur colloid only represents a negligible risk to participants.

Potential benefits: This project has the potential to benefit future breast cancer patients by providing imaging that enhances ability to identify small vessels in the body, thereby decreasing the risk of accidental damage to the vessels and increasing the possibility of effective repair (i.e., anastomosis). Potential benefits to science and society in general include refinement of a surgical technique with the potential to reduce the risk of lymphedema for breast cancer patients undergoing axillary surgery.

7. Data and Safety Monitoring Plan

The PI and study team will be involved in ongoing monitoring for data and safety concerns.

Our proposed phase II, non-blinded clinical trial represents a low risk of adverse events, and the described DSMP is commensurate with this level of risk. The PI and study team will meet weekly to discuss recruitment, accrual and retention of participants. They will also review any safety concerns during these meetings. The study team will discuss data quality monthly.

We feel that the risks to participating in this study are minimal and our research team will be continually reviewing data and subject safety at weekly study team meetings to discuss study progress including recruitment/enrollment, data integrity, and adverse events (AEs). This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for routine toxicity and adverse event (AE) reporting. Incisional pain, breast seromas, and breast/arm swelling are expected events after breast and axillary surgery. We will consider injection site reactions, such as redness of the skin or hives, to be unexpected adverse events. We also consider allergic reactions to the dye (such as cough or difficulty swallowing) to be unexpected adverse events. We will submit any unanticipated adverse effects to sponsor and to the IRB as soon as possible, but no event later than 10 working days after the investigator first learns of the effect.

The focus of this study is to test the feasibility of ICG mapping through the Asimov Imaging Platform. Potential advantages to the Asimov Imaging Platform compared to other means of immunofluorescence detection is that it allows real time imaging without altering ambient light.

Only coded data related to the imaging acquisition will be stored on the OnLume off campus data server. Data will be transferred on an encrypted USB drive to OnLume. After confirmation that the data has transferred and is not been corrupted, the source data will be deleted from the imaging platform. Imaging data will also be stored on the surgery servers using the same method of encrypted USB. No PHI will be stored outside of the UW Department of Surgery secure server (see section that follows).

8. Data and Record Keeping

The PI will manage and oversee all study activities including the collection and protection of research data. All UW-Madison personnel and external collaborators involved in the study will have completed University-provided human subjects and HIPAA training. The study staff is trained to protect research documents and all precautions will be taken to keep records confidential. Any identifying personal information collected during the project will be kept secure by the research team in accordance with IRB requirements. Only UW investigators and key personnel will have access to these files with PHI.

Imaging data will be coded with a unique sample ID # (e.g., 123) and will be stored on the secure surgery server and on the OnLume's off-campus data server. Data will be transferred using an encrypted USB drive. No PHI will be stored on the OnLume data server. The subject code is linked to a separate spreadsheet, which lists the subject number and the patient identifiable information including medical record number. The code will be stored separately from the research data on the secure Department of Surgery server. All electronic data is password and firewall security protected. When a patient is imaged through the Asimov Imaging Platform, only the subject ID will be entered into the system. In this way, the imaging will always be "coded" from the time of image acquisition. From the OnLume system, the imaging data will be transferred using an encrypted USB hard drive. Once data is confirmed to be successfully transferred to OnLume and the secure Dept. of Surgery server, it will be permanently deleted from the imaging platform.

Datasets containing patient identifiers will not be copied or shared with others for analysis. Only datasets without identifiers can be shared for analysis. Upon completion of this research, the link to identifiable information will be destroyed, permanently de-identifying the data.

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