2 TITLE: A Randomized Clinical Trial of the LYMPHA procedure for the Prevention of Lymphedema after Axillary Lymphadenectomy

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Coordinating Center
Stanford Cancer Institute
875 Blake Wilbur Drive
Stanford, CA 94305

Protocol Director
Dung Nguyen MD PharmD
770 Welch Road, Suite 400
Palo Alto, CA 94304
(650) 725-2766
(650) 725-6605
nguyendh@stanford.edu

Co-Investigators
Irene Wapnir MD
300 Pasteur Drive H3625A
(650) 721-5705
(650) 736-1163
Palo Alto, CA 94305
wapnir@stanford.edu

Mardi Karin, MD
2589 Samaritan Drive
San Jose, CA 95124
(408) 828-0525
mkarinmd@stanford.edu

Frederick Dirbas, MD
875 Blake Wilbur Drive, CC2236
Stanford, CA 94305
(650) 723-5672
dirbas@stanford.edu

Jacqueline Tsai, MD
900 Blake Wilbur Drive, MC 5348
Palo Alto, CA 94304
(650) 498-6004
jatsai@stanford.edu

Kimberly Stone, MD
2589 Samaritan Drive
San Jose, CA 95124
(408) 426-4900
kstone@stanford.edu

Biostatistician
Hao He
870 Quarry Road
Palo Alto, CA 94304-5407
650-725-3822
hhe7@stanford.edu

Michael Januszyk, MD PhD
257 Campus Dr. GK-102
Stanford, CA 94305
773-610-0526
januszyk@stanford.edu

Study Coordinators
Shannon Meyer, CCRC
770 Welch Rd., Ste 400
Palo Alto, CA 94304
(650) 721-1807
Smeyer27@stanford.edu

David Perrault, MD
770 Welch Road, Suite 400
Palo Alto, CA 94304
(650) 725-2766
perrault@stanford.edu

Kassandra Carrion
770 Welch Road, Suite 400
Palo Alto, CA 94304
(650) 505-7749
kcarrion@stanford.edu

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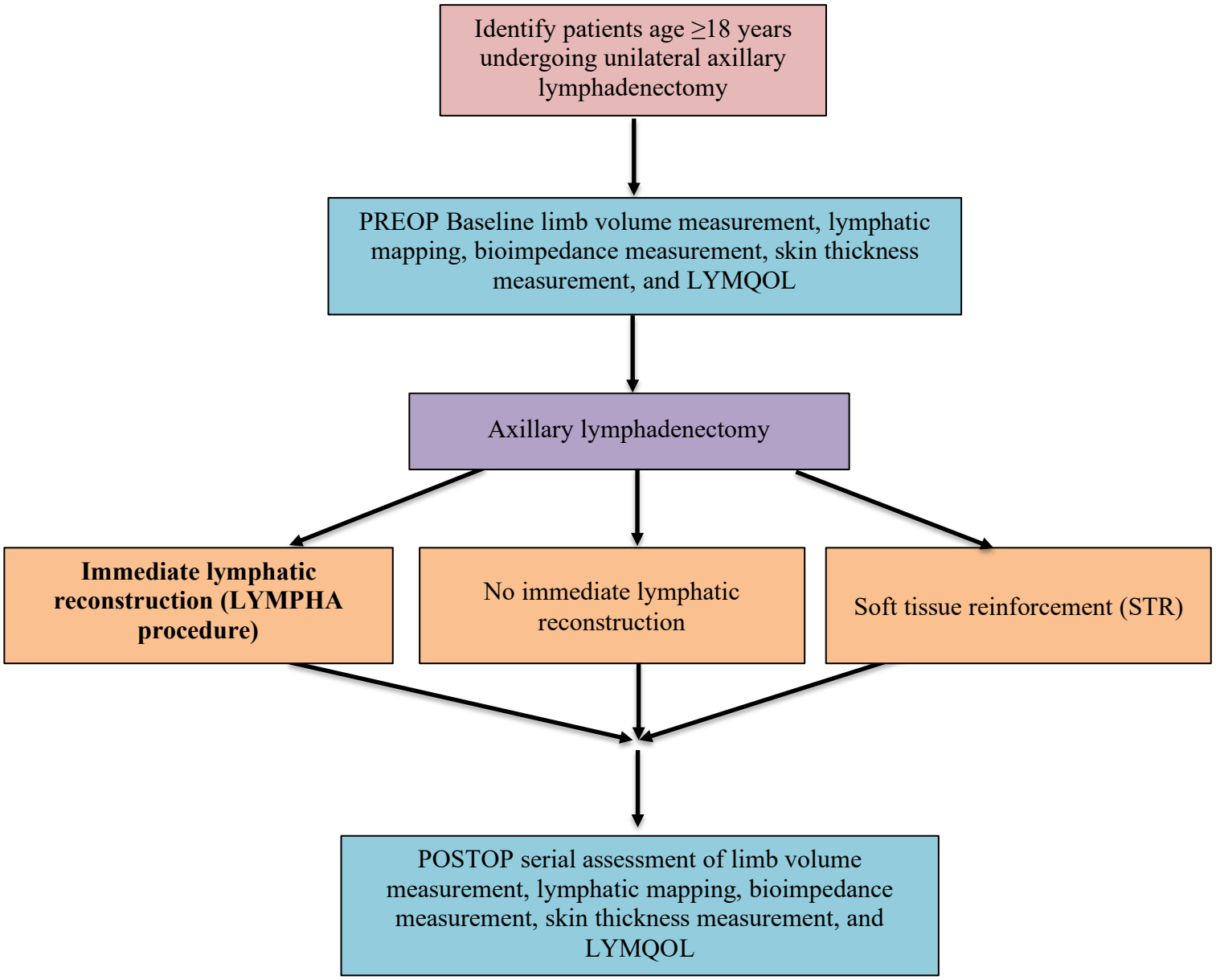
PROTOCOL SYNOPSIS

TITLE	A Randomized Clinical Trial of the LYMPHA procedure for the Prevention of Lymphedema after Axillary Lymphadenectomy
STUDY PHASE	Randomized Clinical Trial
INDICATION	Unilateral axillary lymphadenectomy related to breast cancer
INVESTIGATIONAL PRODUCT OR PROCEDURE	Immediate lymphatic reconstruction (LYMPHA procedure)
PRIMARY OBJECTIVE(S)	To determine if the use of LYMPHA in breast cancer patients undergoing axillary lymphadenectomy will reduce the risk of limb lymphedema as determined based on the fulfilment of at least one of the following three criteria: (1) More than 10% increase in the relative excess volume of the affected limb; (2) More than 50% time delay of ICG migration from the injection points to the axilla of the affected upper limb in comparison with the contralateral healthy upper limb; or (3) Aggravation of one or more stages in Yamamoto's classification of the dermal back flow and morphology of the lymphatic collectors.
SECONDARY OBJECTIVE(S)	To determine if the use of LYMPHA in breast cancer patient undergoing axillary lymphadenectomy will reduce the risk of limb lymphedema as measured by other metrics of clinical lymphedema, including limb volume, skin thickness measurements, and bioimpedance spectroscopy. To determine if the use of LYMPHA in breast cancer patients undergoing axillary lymphadenectomy will improve quality of life, as measured by limb-lymphedema-specific quality of life (LYMQOL) tool.
TREATMENT SUMMARY	Patients undergoing unilateral axillary lymphadenectomy will undergo the immediate lymphatic reconstruction (LYMPHA) at the same time.
SAMPLE SIZE	100 patients (randomized)
STATISTICAL CONSIDERATIONS	Fisher's exact test and general linear mixed models

SCHEMA



Key:
LYMQOL = limb-lymphedema-specific quality of life
LYMPHA = LYmphatic Microsurgical Preventive Healing Approach

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

LYMQOL	limb-lymphedema-specific quality of life
LYMPHA	LYmphatic Microsurgical Preventive Healing Approach
ICG	Indocyanine Green
ALND	Axillary Lymph Node Dissection

1. OBJECTIVES

1.1. Primary Objective

To determine if the use of LYMPHA in breast cancer patients undergoing axillary lymphadenectomy will reduce the risk of limb lymphedema as measured by ICG lymphangiography and by relative limb volume measurement.

1.2. Secondary Objectives

To determine if the use of LYMPHA in breast cancer patient undergoing axillary lymphadenectomy will reduce the risk of limb lymphedema as measured by other metrics of clinical lymphedema, including limb volume, skin thickness measurements, bioimpedance spectroscopy. To determine if the use of LYMPHA in breast cancer patients undergoing axillary lymphadenectomy will improve quality of life, as measured by limb-lymphedema-specific quality of life (LYMQOL) tool.

2. BACKGROUND

2.1 Study Disease

Lymphedema is a chronic, progressive, and debilitating condition that occurs with disruption or obstruction of the lymphatic system. Normally, lymphatics drain fluid from the interstitium that is not reabsorbed by venous capillaries [1]. When this drainage system doesn't function, lymphatic fluid accumulates, causing edema. Ultimately, this results in a limb that is heavy, painful, less functional, and at greater risk of infection and even malignant transformation [2]. Additionally, lymphedema significantly decreases quality of life [3, 4].

Lymphedema can be congenital (primary) or acquired (secondary). Secondary, or acquired, lymphedema is more common than primary lymphedema [1]. Worldwide, the most common cause of secondary lymphedema is infection and subsequent obstruction of the lymphatic system by *Wuscheria banrofti* [5]. In the United States, upper extremity lymphedema is commonly the result of the surgical and radiation therapy for breast cancer [2]. A large meta-analysis elucidated that roughly 1 in 5 women that survive breast cancer in North America will develop lymphedema [6]. Reported risk factors for developing lymphedema after sentinel lymph node biopsy (SLNB) is 3-6%, after axillary lymph node dissection (ALND) is 16-27% and as high as 40% after ALND and radiotherapy [6].

The standard of care for lymphedema continues to be conservative therapy, which includes manual decongestive therapies and compressive bandaging. Unfortunately, these conservative therapy modalities are inconvenient and tend to result in poor patient adherence [7].

Surgical options for lymphedema include physiologic surgeries and de-bulking procedures. Physiologic lymphedema surgery attempts to restore anatomic lymphatic flow. Specifically, this includes lymphovenous bypass and vascularized lymph node transfer. Physiologic surgeries are

especially effective if performed before there is chronic fibrotic change in the affected limb [8]. Debulking procedures, such as suction assisted lipectomy and the modified Charles procedure are more suited for late stage lymphedema, after the lymphedematous tissue has remodeled, producing a large fibrotic component [9]. Unfortunately, these are highly morbid and mandate lifetime use of compression garments to prevent redevelopment of lymphedema [10].

Currently, there is no cure for lymphedema and the available therapeutic options are less than satisfying, prompting the investigation of prophylactic measures. Immediate lymphatic reconstruction, the LYMPHA (Lymphatic Microsurgical Preventative Healing Approach) technique, is one promising prophylactic intervention.

2.2 Study Agent/Device/Procedure

Immediate lymphatic reconstruction, termed LYMPHA or Lymphatic Microsurgical Healing Approach, involves anastomosing lymphatics to nearby veins distal to the site of injury to facilitate normal lymphatic drainage [11]. LYMPHA was first described by Boccardo et al [11]. In their series of 74 patients who underwent immediate lymphatic reconstruction at the time of axillary lymph node dissection (ALND), the reported rate of lymphedema was 4% at 4 years [12]. This is in contrast to the reported incidence of lymphedema after axillary lymph node dissection alone, which ranges from 13-65% [13-16].

These initial findings have since been supported by other groups. Hahamoff et al decreased their institution rate of lymphedema after ALND from 40% to 12.5% when paired with LYMPHA [17]. Feldman et al reported a 12.5% lymphedema rate after ALND and LYMPHA [18]. Johnson et al recently performed a meta-analysis to assess the impact of LYMPHA after ANLD and found that in incidence of lymphedema after ALND and radiation was 33.4% compared to 10.3% in those who underwent ALND, radiation, and LYMPHA [19].

The body of the referenced data suggests that LYMPHA is a viable modality for the prevention of lymphedema. However, to date, there are no randomized clinical trials evaluating the efficacy and safety of this procedure. Therefore we suggest initiating a randomized clinical trial to more accurately elucidate the role of the LYMPHA procedure in the prevention of lymphedema after axillary lymphadenectomy.

For clinicaltrials.gov compliance
The LYMPHA procedure is not a drug so this does not apply.

2.3 Rationale

As reviewed in sections 2.1 and 2.2, lymphedema is a progressive and debilitating disease that significantly affects quality of life. Current therapeutic options for lymphedema are less than optimal. Manual decompressive therapy is highly inconvenient with poor patient adherence, physiologic surgeries are most effective in early lymphedema, and debulking procedures are morbid. Given that 1 in 5 women tend to develop lymphedema after surviving breast cancer, a prophylactic therapy would be advantageous. The LYMPHA procedure is one prophylactic modality that has promising results as reviewed above. We propose a clinical trial to more

accurately evaluate the role of LYMPHA in preventing lymphedema after axillary lymphadenectomy.

2.4 Study Design

We propose a randomized clinical trial to determine the efficacy of the LYMPHA procedure in breast cancer patients undergoing axillary lymphadenectomy.

Up to 100 subjects will be enrolled in the trial; they will undergo either lymphadenectomy, lymphadenectomy with soft tissue reinforcement (STR) or lymphadenectomy with LYMPHA. Subjects must meet all additional defined inclusion and exclusion criteria.

Please note that soft tissue reinforcement is these patients is common practice and used as the standard of care in some lymphedema centers. The addition of this group is important because it allows a comparison of LYMPHA to a currently commonly practiced alternatice and the ability to assess for superiority of the LYMPHA procedure. In short, at Stanford, we perform soft tissue reinforcement with collagen scaffolding commonly and would like to start including these patients in our prospective analysis/study.

All patients that are enrolled will be randomized into one of three groups: Group A (surgical control group 1) – the patients will undergo lymphadenectomy alone with simple wound closure, Group B (surgical control group 2) – the patients will undergo lymphadenectomy with soft tissue reinforcement using a collagen scaffold, or Group C (treatment group) - the patients will undergo lymphadenectomy and LYMPHA at the time of wound closure as an adjunct to lymphadenectomy procedure.

Randomization will occur after it has been determined that patients are eligible for the trial intraoperatively. Patients are deemed eligible for the trial intraoperatively if ALND is performed and there are available lymphatics to bypass.

The study will be double blind and randomized. Each patient will be randomized to either the treatment or control group 1 or 2 and will be blinded to their group. While the surgeons will not be blinded, all personnel who track outcomes in clinic will be blinded. The primary outcomes that the protocol is designed to evaluate include efficacy, safety, and impact on the quality of life.

The study was started with 2-arm design (Group A and Group C). A preliminary assessment to the trial effects will be conducted using the current collected data. After the revised protocol is approved, the study will recruit subjects in the new 3-arm phase: 90 subjects (30 subjects per arm in the three groups (Groups A, B, C)

2.5 Correlative Studies Background

We plan to investigate the use of the LYMPHA procedure in decreasing the risk for lymphedema and improving quality of life after axillary lymphadenectomy. As reviewed in section 2.2, this procedure has been shown to reduce the incidence of lymphedema compared to ALND alone in prospective cohort studies, however, no randomized clinical trial has been conducted to confirm this observation.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

3.1.1 Ages 18 to 75 years (inclusive)

3.1.2 Patients undergoing unilateral breast cancer related axillary lymphadenectomy

3.1.3 Free of distant metastasis in preoperative screening

3.1.4 Histology results of axillary lymph nodes could be either Negative or Positive

3.1.5 Patients who undergo preoperative chemotherapy can be included

3.1.6 Willingness and ability to provide written informed consent

3.1.7 Willingness and ability to comply with all study procedures

3.1.8 Preauthorization for ALND with LYMPHA is received

3.1.9 ALND is performed during surgery and there are available lymphatics for bypass

3.2 Exclusion Criteria

3.2.1 Primary lymphedema of the affected upper limb

3.2.2 Secondary lymphedema of the affected limb prior to the lymphadenectomy

3.2.3 Radiotherapy at the axilla before the study / surgery

3.2.4 Allergic reaction to porcine collagen or ICG

3.2.5 Receiving radiation therapy to the involved nodal basin in a period less than 4 weeks after the surgery

3.2.6 Concurrent participation in a clinical trial of any other investigational drug or therapy, regardless of indication, within 1 month before screening

3.2.7 Other medical condition that could lead to limb edema, such as (but not limited to primary lymphedema or acute venous thrombosis

3.2.8 Other medical condition that could result in symptoms overlapping those of lymphedema in the affected limb (e.g., pain, swelling, decreased range of motion)

3.2.9 Either of the following, at the time of baseline evaluation: ipsilateral:contralateral limb volume ratio>1.1 or R0 bioimpedance ratio > 1.106 when the nondominant limb is at risk, and 1.134 when the dominant limb is at risk.

3.2.10 Life expectancy < 2 years for any reason

3.2.11 Pregnancy or nursing

3.2.12 Substance abuse (such as alcohol or drug abuse) within 6 months prior to screening

3.2.13 Severe psychiatric disease

3.2.14 Significant or chronic renal insufficiency (defined as serum creatinine > 2.5 mg/dL or an estimated glomerular filtration rate [eGFR] < 30 mL/min at screening) or requires dialytic support

3.2.15 Hepatic dysfunction, defined as alanine transaminase (ALT) or aspartate transaminase (AST) levels > 3 × upper limit of the normal range (ULN) and/or bilirubin level > 2 × ULN at screening

3.2.16 Absolute neutrophil count < 1500 mm3 at screening

3.2.17 Hemoglobin concentration < 9 g/dL at screening

3.2.18 Patients without available lymphatics will be deemed unable to receive LYMPHA procedure.

3.2.19 Any reason (in addition to those listed above) that, in the opinion of the investigator, precludes full participation in the study

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Randomization Procedures

Since this study was started with two groups (A and C), and randomized 13 patients to the control group A and 14 patients to the treatment group C. However, 7 of the 14 patients that were randomized to treatment group C did not receive LYMPHA surgery , were considered screen fails. 27 patients will be used in intent-to-treat (ITT) analysis, however, 7 screen fails will not be counted in the per protocol (PP) analysis. In the new 3-arm phase, the randomization procedures will happen during ALND surgery time. and 90 subjects will be allocated in a 1:1:1 randomization ratio into three groups. The clinic staff obtaining measurements will be blinded to the treatment group

3.5 Study Timeline

Primary Completion:
The study will reach primary completion 24 months from the time the study opens to accrual.

Study Completion:
The study will reach study completion 36 months from the time the study opens to accrual (i.e., 2 year accrual and an additional 12 months to monitor recovery). Length of follow-up for each patient will be 12 months.

4. TREATMENT PLAN

All consenting patients presenting to breast oncologist's or Dr. Nguyen's clinics who are candidates for breast cancer related axillary lymphadenectomy and satisfy the above inclusion and exclusion criteria will be given the option of study enrollment. Patients will be consented to treatment by either Dr. Nguyen or another physician that has met the appropriate prerequisite criteria for peforming LYMPHA.

After informed consent is obtained, all subjects will undergo an initial study evaluation to establish the pre-operative baseline and to determine eligibility for randomization. Group A will undergo axillary lymphadenectomy alone, Group B will undergo axillary lymphadenectomy with soft tissue reinforcement using collagen scaffold, and Group C will undergo axillary lymphadenectomy with immediate lymphatic reconstruction (LYMPHA) using reverse mapping with the SPY System. Each of the baseline measures will be repeated over the 12 months of enrollment in the study according to the STUDY CALENDAR.

1. Indocyanine green lymphography: One of the most important advances in lymphedema staging and in lymphatic vessels detection is indocyanine green (ICG) lymphography [23-27]. In the early lymphedema stages this exam allows to detect percutaneously the lymphatic vessels of the whole limb, up to about 1 cm in depth from the skin surface, by an infrared camera visualization system after intra-dermal injection of ICG in the hand [24, 27]. The change in the lymphatic pattern and reduction in the ICG velocity [26] will be assessed. The healthy upper limb lymphatic pattern will be used as a baseline for each patient. A delay of more than 50% in ICG migration from the injection points to the axilla of the affected upper limb in comparison with the contralateral healthy upper limb will be used as an indication of lymphedema.

2. Limb volume. The volume of the at-risk and the contralateral arm of each subject will be measured through serial quantitation of the limb circumference at 4 cm intervals along its long axis. The procedure is performed with gauged tape to ensure a uniform stretching force. Using these measurements, the volume of the limb is calculated with the truncated cone approximation [20]. Pre- and post-treatment measurements for each subject will be performed by the same operator who, in all cases, will be blinded to the patients' treatment status and to pre-treatment measurement values at the time of the post-treatment assessment. The measured limb volumes at each evaluation will be utilized to generate two data points: the volume V_r of at-risk arm and the volume V_c of contralateral arm. These data will be used to determine a relative excess volume $E=((V_r - V_c)/V_c)\cdot 100\%$ for each patient at the time of measurement [32]. Change in the relative

excess volume will be determined at month 12 as $(E_{12}/E_0 - 1)\cdot 100\%$, where E_0 is the relative excess volume at baseline (t=0) and E_{12} is the relative excess volume at 12 months (t=12).

3. Skin thickness measurements: These will be performed with a Lange skinfold caliper (Beta Technology, Santa Cruz, CA). For each subject, at each assessment, three measurements will be obtained of both upper extremities: the dorsum of the hand; the midpoint of the volar aspect of the forearm; and the midpoint of the medial aspect of the upper arm. At the initial evaluation, a dermatographic pencil is used to mark the site of each measurement. Once the locations are determined, the exact distance of each location is measured from the wrist. These locations are re-utilized for serial measurements during the trial. The calipers will be calibrated prior to each use. The serial evaluations will be performed by the same operator for each subject, and the operator will be blinded to treatment status and to prior measurement values at the time of each assessment. Each reported skin thickness value represents the arithmetic sum of the three measurements/limb at that assessment. The change in the ratio of ipsilateral:contralateral skin thickness will be analyzed as a secondary endpoint.

4. Bioimpedance spectroscopy (BIS) will be performed with the Impedimed SFB7. A four-electrode configuration is used to non-invasively assess the extracellular and intracellular fluid contents of the limb [21]. Data were analyzed according to Cole theory [22], using the manufacturer's software (Impedimed Ltd.), to provide values for R0, the resistance of the extracellular fluid, including lymph, Roo the resistance of total tissue fluid, and Ri, the resistance of the intracellular fluid. For the purposes of these investigations, the ratio of R0 in the ipsilateral:contralateral limbs will be analyzed in each patient as a secondary endpoint.

5. Quality-of-life will be serially assessed, at baseline and at 12-months, using a validated instrument, the LYMQOL [28]. The LYMQOL is divided into four domains: Function, Appearance, Symptoms, and Mood, as well as yielding an overall quality-of-life score. These continuous variables will be analyzed as a secondary endpoint for intergroup comparisons.

A video recording of 1-2 cases in the study group may occur. The purpose of these videos would be to demonstrate the operative method. These recordings may also supplement eventual publications and scientific presentations. Only patients consenting to video recording will be affected. All videos will be deidentified (no facial identification possible). Videos will be kept in a secure, encrypted location and destroyed within 5 years following completion of the study. Photography will be routinely used pre- and postoperatively, with appropriate patient consent, in order to document aesthetic outcomes. Tissue samples will not be retained for future research.

4.1 General Concomitant Medication and Supportive Care Guidelines

There are no known interactions of medications with the intraoperative use of indocyanine green, which we routinely use to assess nipple perfusion during mastectomy. Patients with an allergy to iodine will be excluded. Concomitant radiotherapy is an exclusion criterion for study participation.

Procedures performed are a normal part of clinical management for this condition. Patients in all three groups are subject to the baseline risks of surgical intervention, including but not limited to

bleeding, infection, scar, injury to surrounding structures, risks of anesthesia, and need for further surgery.

4.2 Criteria for Removal from Study

Patients would be removed from the treatment plan in the event of patient withdrawal of consent, allergy, or other unforeseen deleterious events. The patients randomized to the intervention but did not receive the intervention due to unforeseeable reasons will be removed from the study and then replaced.

4.3 Alternatives

Patients will be monitored at routine clinic follow-up visits. They are counseled on warning signs that should prompt a call to their physician, and provided with a phone number at which they can reach a physician for advice at all hours. None of these methods of protection jeopardizes patient confidentiality beyond the risks inherent in routine patient care. A study participant may undergo the alternative (i.e., forego LYMPHA) if she chooses to withdraw from the study.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent/Device/Procedure

As described in section 2.2, the intervention arm involves the use of the LYMPHA procedure at the time of axillary lymphadenectomy, specifically, anastomosing lymphatics to nearby veins distal to the site of injury to facility normal lymphatic drainage [11]. This procedure is a normal part of clinical management for this condition. The purpose of this study is to more accurately elucidate the efficacy and role of this procedure. Nanofibrillar collagen scaffolds are FDA approved product for soft tissue reinforcement and is routinely used for this indication at the surgical site to enhance tissue repair.

5.2 Availability

No applicable

5.3 Agent Ordering

Not applicable

5.4 Agent Accountability

Not applicable

6. DOSE MODIFICATIONS

Not applicable

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Potential risks of LYMPHA include the baseline risks of surgical intervention, including but not limited to bleeding, infection, scar, injury to surrounding structures, risks of anesthesia, and need for further surgery. The investigation, as designed, is deemed to have a low potential for risk. Only cut lymphatics will be repaired. The LYMPHA procedure is used now in several clinical centers without reported complications [12, 19, 29].

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v4.03. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. All Serious Adverse Events (SAEs) will be tracked until resolution, or until 30 days after the last dose of the study treatment.

SAEs CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

8. CORRELATIVE/SPECIAL STUDIES

There are no planned correlative studies at this time

9. STUDY CALENDAR

	Pre-Study	Intervention	Intervention follow-up	Post-op Wk 1[c]	Post-op Wk 24[d]	Post-op Wk 36 optional [d]	Post-op Wk 48[d]	Off Study[b]
Axillary Lymphadenectomy alone, Axillary Lymphadenectomy with STR OR Axillary Lymphadenectomy + LYMPHA[a]		X						
Informed consent	X							
Demographics	X							
Medical history	X							
Physical exam	X		X	X	X	X	X	
Vital signs[f]	X	X	X	X	X	X	X	
Height	X		X	X	X	X	X	
Weight	X		X	X	X	X	X	
LYMQOL Patient questionnaire	X					X	X	
Limb volume	X					X	X	
Skin Thickness Measurements	X					X	X	
Bioimpedance Spectroscopy (LDex)	X					X	X	
ICG Lymphograhy		X				X	X	
Adverse event evaluation		X ----------X						

a: Investigation: LYMPHA procedure
b: Off-study evaluation.
c. +/- 1 week
d: +/- 4 weeks
e: demographics refers to first name, last name, MRN, DOB
f: vital signs include BP and HR, with RR and temperature as optional vital signs

10. MEASUREMENTS

10.1 Primary outcome measure: Incidence of lymphedema

The primary endpoint in this study is a binary outcome variable defined as the presence of lymphedema at Month 12 ("Yes"/"No"), based on the fulfilment of at least one of the following three criteria: (1) More than 10% increase in the relative excess volume of the affected limb; (2) More than 50% time delay of ICG migration from the injection points to the axilla of the affected upper limb in comparison with the contralateral healthy upper limb; or (3) Aggravation of one or more stages in Yamamoto's classification of the dermal back flow and morphology of the lymphatic collectors.

The efficacy of the LYMPHA procedure will be determined by comparison between the LYMPHA group (Group C) and lymphadenectomy group (Group A).

10.2 Secondary Outcomes: Other metrics of lymphedema

10.2.1 The comparison of lymphedema rates in the three groups by pairwise comparison.

10.2.2 The ratio of ipsilateral:contralateral skin thickness measured by large skinfold calipers

10.2.3 The ratio of R0 in the ipsilateral:contralateral limbs measured using BIS

10.2.4 The excess volume pairwise comparison between the LYMPHA group and each of the two control groups. We will also compare the excess volume between the two control groups.

10.3 Secondary Outcome: Quality of life

10.3.1 LYMQOL Total score

10.3.2 Measurement time points will be at postoperative clinic visits, which will occur at 1 week and every 3 months for a duration of 12 months.

10.3.3 Response is not the primary outcome

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g, advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director and her research team, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the REDCap system. The REDCap system is encrypted and compliance for PHI per Stanford University Regulations.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

The objectives of the study are to assess the efficacy of the LYMPHA procedure when compared to the results of wound closure alone and wound closure with soft tissue reinforcement in similar clinical cohorts. We plan to enroll a total of 100 patients in this randomized parallel clinical trial.

The hypothesis to be tested is that adjunctive surgical use of LYMPHA with an axillary lymphadenectomy will produce a superior effect in terms of lymphedema prevention when compared to the effect of an axillary lymphadenectomy alone and an axillary Axillary lymphadenectomy with STR. The primary endpoint, incidence of lymphedema at month 12, will be compared between LYMPHA and the control, axillary lymphadenectomy alone arm using a significance level of 5%.

12. 1 .1 Randomization

Since this study was started with two groups (A and C), and randomized 13 patients to the control group A and 14 patients to the treatment group C. However, 7 of the 14 patients that were randomized to treatment group C did not receive LYMPHA surgery, were considered screen fails, Therefore in the preliminary assessment, 27 patients will be used in intent-to-treat (ITT) analysis, however, 7 screen fails will not be counted in the per protocol (PP) analysis. In the new 3-arm phase, the randomization procedures will happen during ALND surgery time. and 90 subjects will be allocated in a 1:1:1 randomization into three groups. The clinic staff obtaining measurements will be blinded to the treatment group.

12.2 Preliminary assessment

Since the study has stopped 2-arm recruitment and will start the new 3-arm recruitment, the current collected 2-arm data will be used to conduct a preliminary assessment, to check the comparability, safety and efficacy of the trial.

Preliminary assessment will evaluate the primary endpoint (presence of lymphedema) and secondary endpoints (skin thickness, BIS, and LYMPQOL). Both intent-to-treat (ITT) analysis and per protocol (PP) analysis will be explored.

12.2 Summary of analyses

Preop visit: After obtaining written consent, medical history, current medications, and allergies will be reviewed, height/weight/VS, physical exam by PI will be conducted, limb measurements performed (volume, BIS, skin thickness), ICG lymphography, and LYMPQOL survey completed.

T1 (1 week following surgery): physical exam, VS, height, weight, photos, symptoms/AE review.

T2 (6 months following surgery): physical exam, VS, height, weight, photos, symptoms/AE review.

T3 (Optional 9 months following surgery): physical exam, measurements (volume, BIS, skin thickness), ICG lymphography, LYMPQOL survey, VS, height, weight, photos, symptoms/AE review.

T4 (12 months following surgery): physical exam, measurements(volume, BIS, skin thickness), ICG lymphography, LYMPQOL survey, VS, height, weight, photos, symptoms/AE review.

Primary endpoint (rate of lymphedema based on volume analysis and ICG analysis) and secondary endpoints (BIS, skin thickness, ICG lymphography and LYMPQOL survey) will be evaluated at final analysis.

12.3 Descriptive Statistics and Exploratory Data Analysis

Preliminary analyses will include graphical methods to check for outliers to identify errors and highly unusual values and to check assumptions needed for analysis, such as normality. Normally distributed variables will be summarized by mean and standard deviation and compared between the three treatment arms using ANOVAs. Non-normally distributed variables will be summarized by median and interquartile range and compared using the Kruskal Wallis test.

12.4 Primary Analysis

The efficacy of the LYMPHA procedure will be determined by the comparison of the lymphedema rates in the LYMPHA group and the lymphadenectomy alone control group, and by the comparison of the lymphedema rates in the LYMPHA group and the lymphadenectomy with STR control group

12.4.1 Analysis Population

All participants will be included in ITT (intent to treat) analysis. Participants who had screen fail will not be included in PP (per protocol) analysis. Missing data and non-adherence to protocol will be reported and its impact will be analyzed.

12.4.2 Analysis Plan

Fisher's exact tests will be used to determine significance of differences in lymphedema rates between LYMPHA and the lymphadenectomy alone control arm, and significance of differences in lymphedema rates between LYMPHA and the lymphadenectomy with STR control arm. If treatment arms differ significantly at baseline in demographics (for e.g. age), lymphedema status at the end of treatment will be compared between treatment groups via a logistic regression, using the necessary covariates. Depending on the attrition rate, we may also use mixed effects logistic regression models to determine whether the treatment arms significantly differ in the binary endpoint (lymphedema status). Mixed models automatically handle missing data, producing unbiased estimates as long as observations are missing at random. This allows the use of all available data from all subjects, thereby minimizing the effects of loss to follow-up.

12.5 Secondary Analysis

Secondary endpoints include skin thickness, BIS and quality of life as measured by the LYMQOL. We will also compare the lymphedema rates in the three groups by pairwise comparison as a secondary analysis.

12.5.1 Analysis Population

All participants will be included in these secondary ITT analyses. Participants who had screen fail will not be included in secondary PP analysis. Any missing data will be reported as such in the results.

12.5.2 Analysis Plan

All secondary outcome measures (the ratio of at-risk: contralateral skin thickness; ratio of R0 in the at-risk: contralateral limbs; and total LYMQOL score) are continuous scores and will rely on generalized linear mixed models (GLMMs). GLMMs properly account for correlations induced by repeated measurements within subjects and automatically handle missing data, as noted above. To make the most efficient use of our data we will fit a single model using all time points for each outcome. To allow for the possibility that the outcome may not have a linear relationship with time, we will treat time as a categorical variable. Treatment group and time main effects, along with two-way interactions of time and group, will be used as independent variables and the time by group interaction term will be used to determine the statistical significance of the difference in the outcome between the LYMPHA group and control groups.

The primary analysis will use all subjects enrolled and assigned to treatment, categorized by their planned treatment (intention to treat, ITT). A sensitivity analysis will use the set of subjects enrolled and treatment-assigned in the study who completed the study protocol, categorized according to treatment actually received (per protocol, PP).

12.6 Sample Size

12.6.1 Accrual estimates

100 participants will be recruited prospectively. Ample time has been allotted for patient recruitment. In the unlikely event that there is difficulty in recruiting a sufficient number of patients at Stanford Hospital, additional recruitment efforts at affiliated Stanford sites will be considered, if approved.

12.6.2 Sample size justification

The risk of lymphedema development is considered to be 30% in the surgical control group (group A), and studies have shown it is to be reduced to 4% when LYMPHA is applied during an axillary lymphadenectomy [12, 30]. With N=40 subjects (20 subjects per arm A and C) in the first stage, the power to stop after the first stage is 56%. Finally, with additional N=40 subjects (20 subjects per arm A and C) in the second stage, the overall power to stop at either the first or the second stage is 88%.

12.6.3 Effect size justification

The effect size used in the previous subsection was based on the study conducted by Boccardo et al, in which 74 patients underwent LYMPHA procedure after axillary lymph node dissection [12]. The incidence of lymphedema in the cited cohort after the LYMPHA procedure was 4.05%. Comparatively, the incidence of lymphedema after axillary lymph node dissection ranges from 13-65% [13-16]. This study provides a reasonable baseline for comparison to as it includes the same target population (women undergoing axillary lymphadenectomy related to breast cancer) and shows a substantially lower incidence of lymphedema compared to other literature.

12.7 Criteria for future studies

The criteria for success of the LYMPH procedure would include: the metrics of lymphedema (limb volume, skin thickness, bioimpedance spectroscopy, lymphatic pattern) and quality of life will be improved compared to the control group ($p<0.05$). Additionally, the complication rate after the LYMPHA procedure will be comparable or less than axillary lymphadenectomy alone ($p<0.05$).

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APPENDICES

APPENDIX A**: Participant Eligibility Checklist**

Protocol Title:	A Randomized Clinical Trial of the LYMPHA procedure for the Prevention of Lymphedema after Axillary Lymphadenectomy
Protocol Number:	TBD
Principal Investigator:	Dung H Nguyen, MD PharmD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. Ages 18 to 75 years (inclusive)	☐	☐	
2. Patients undergoing unilateral breast cancer related axillary lymphadenectomy	☐	☐	
3. Free of distant metastasis in preoperative screening	☐	☐	
4. Histology results of axillary lymph nodes could be either Negative or Positive	☐	☐	
5. Patients who undergo preoperative chemotherapy can be included	☐	☐	
6. Willingness and ability to provide written informed consent	☐	☐	
7. Willingness and ability to comply with all study procedures	☐	☐	
8. Preauthorization for ALND with LYMPHA is received	☐	☐	
9. ALND is performed during surgery and there are available lymphatics for bypass	☐	☐	

Exclusion Criteria (From IRB approved protocol)			
1. Primary lymphedema of the affected upper limb	☐	☐	
2. Secondary lymphedema of the affected limb prior to the lymphadenectomy	☐	☐	
3. Radiotherapy at the axilla before the study / surgery	☐	☐	
4. Allergic reaction to porcine collagen or ICG	☐	☐	
5. Receiving radiation therapy to the involved nodal basin in a period less than 4 weeks after the surgery	☐	☐	
6. Concurrent participation in a clinical trial of any other investigational drug or therapy, regardless of indication, within 1 month before screening	☐	☐	
7. Other medical condition that could lead to limb edema, such as (but not limited to primary lymphedema or acute venous thrombosis	☐	☐	
8. Other medical condition that could result in symptoms overlapping those of lymphedema in the affected limb (e.g., pain, swelling, decreased range of motion)	☐	☐	
9. Either of the following, at the time of baseline evaluation: ipsilateral:contralateral limb volume ratio>1.1 or R0 bioimpedance ratio > 1.106 when the nondominant limb is at risk, and 1.134 when the dominant limb is at risk.	☐	☐	
10. Life expectancy < 2 years for any reason	☐	☐	
11. Pregnancy or nursing	☐	☐	
12. Substance abuse (such as alcohol or drug abuse) within 6 months prior to screening	☐	☐	
13. Severe psychiatric disease	☐	☐	
14. Significant or chronic renal insufficiency (defined as serum creatinine > 2.5	☐	☐	

mg/dL or an estimated glomerular filtration rate [eGFR] < 30 mL/min at screening) or requires dialytic support			
15. Hepatic dysfunction, defined as alanine transaminase (ALT) or aspartate transaminase (AST) levels > 3 × upper limit of the normal range (ULN) and/or bilirubin level > 2 × ULN at screening	☐	☐	
16. Absolute neutrophil count < 1500 mm3 at screening	☐	☐	
17. Hemoglobin concentration < 9 g/dL at screening	☐	☐	
18. Any reason (in addition to those listed above) that, in the opinion of the investigator, precludes full participation in the study	☐	☐	

*****All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	
Secondary Reviewer Signature:	Date:
Printed Name:	
Study Coordinator Signature:	Date:
Printed Name:	