Prospective Evaluation of a Surgical Solution for Breast Cancer-Associated Lymphedema

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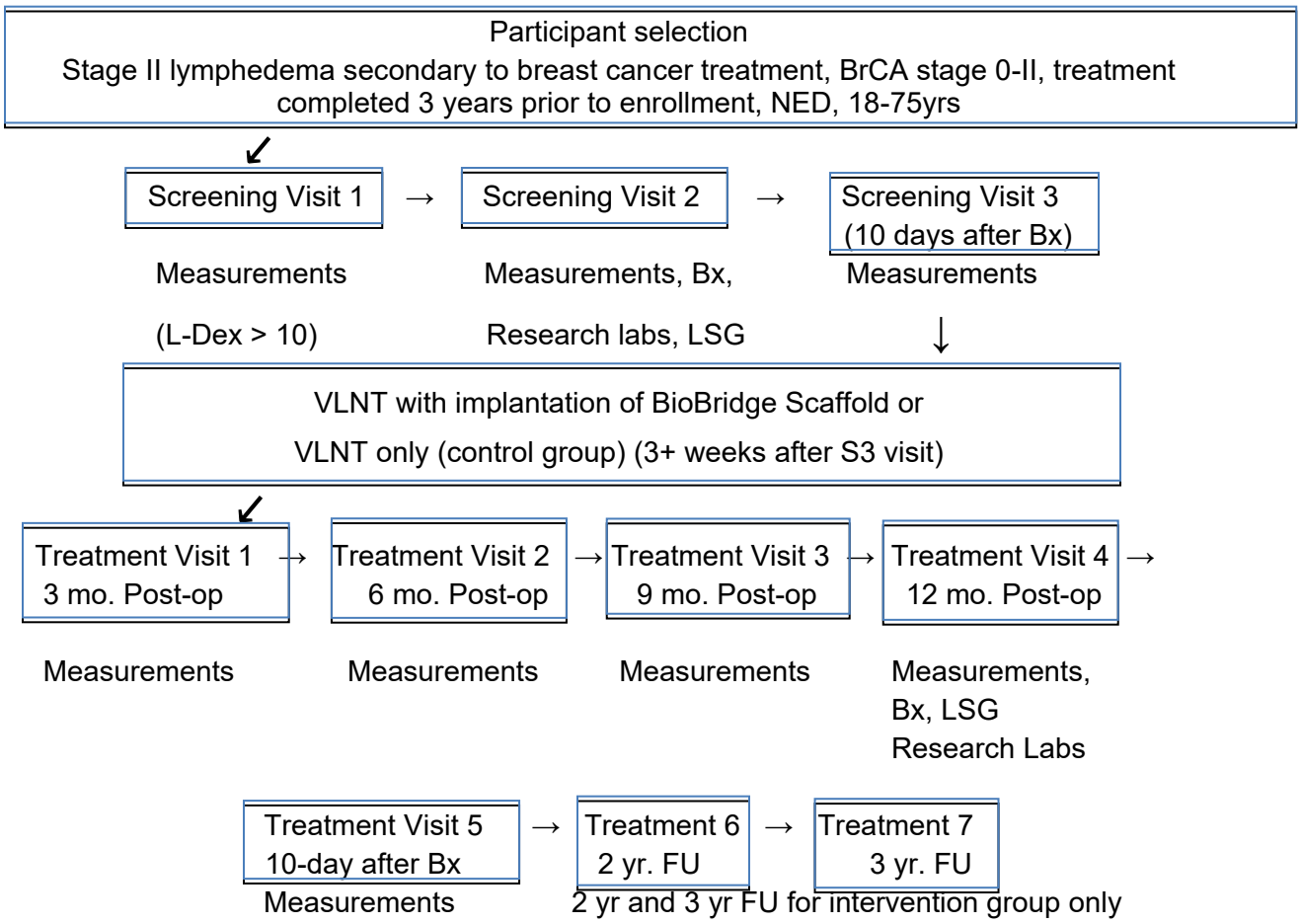
PROTOCOL SYNOPSIS

TITLE	Prospective Evaluation of a Surgical Solution for Breast Cancer-Associated Lymphedema
STUDY PHASE	N/A (randomized, controlled, device study)
INDICATION	Upper extremity lymphedema secondary to breast cancer treatment
INVESTIGATIONAL PRODUCT	BioBridge scaffold
PRIMARY OBJECTIVE(S)	To determine whether the addition of the BioBridge scaffold to vascularized lymph node transfer will improve the outcome of surgical treatment of secondary arm lymphedema resulting from treatment of breast cancer. Primary endpoint is the post-surgical % change in excess limb volume, measured at 12 months following the surgical procedure.
SECONDARY OBJECTIVE(S)	Secondary endpoint is change in measurement of dermal thickness, screening evaluations to Month 12, as measured by caliper skin fold thickness.
TREATMENT SUMMARY	Vascularized lymph node transfer (VLNT) with placement of BioBridge scaffold
SAMPLE SIZE	BioBridge: 48; Control: 12
STATISTICAL CONSIDERATIONS	Open-label, randomized, control trial. This study is open-label due to visibly apparent differences between the recipients of the surgery.

SCHEMA


Participant selection
Stage II lymphedema secondary to breast cancer treatment, BrCA stage 0-II, treatment completed 3 years prior to enrollment, NED, 18-75yrs
Screening Visit 1
Screening Visit 2
Screening Visit 3 (10 days after Bx)
Measurements (L-Dex > 10)
Measurements, Bx, Research labs, LSG
Measurements
VLNT with implantation of BioBridge Scaffold or
VLNT only (control group) (3+ weeks after S3 visit)
Treatment Visit 1 3 mo. Post-op
Treatment Visit 2 6 mo. Post-op
Treatment Visit 3 9 mo. Post-op
Treatment Visit 4 12 mo. Post-op
Measurements
Measurements
Measurements
Measurements, Bx, LSG Research Labs
Treatment Visit 5 10-day after Bx
Treatment 6 2 yr. FU
Treatment 7 3 yr. FU
Measurements
2 yr and 3 yr FU for intervention group only

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ACS	American Cancer Society	IV	Intravenous
ADL	Activities of daily living	LLN	Lower limit of normal
AE	Adverse event	LNF	Lymph node fragments
ALND	Axillary lymph node dissection	LSG	Lymphoscintigraphy
BB	BioBridge	L-Dex	Lymphedema index
BIS	Bioimpedance spectroscopy	LV	Limb volume
BrCA	Breast Cancer	LVA	Lymphaticovenous anastomosis
BSA	Body surface area	LymQoL	Lymphedema Quality of Life
Bx	Biopsy	MFBIA	Multiple Frequency Bioimpedance Analysis
CBC	Complete blood count	NED	No evidence of disease
CDPT/CDT	Completed Decongestive Physiotherapy/Therapy	PLT	Platelet
CDRH	Center for Devices and Radiological Health	PT/PTT	Prothrombin Time/Partial Thromboplastin Time
CI	Confidence interval	RNA	Ribonucleic acid
CMP	Comprehensive metabolic panel	ROI	Region of Interest
CNS	Central nervous system	RR	Response rate
CRF	Case report/record form	SAE	Serious adverse event
CTCAE	Common Terminology Criteria for Adverse Events	SLND	Sentinel lymph node dissection
CXR	Chest x-ray	SOC	Standard of Care
DSMB	Data Safety Monitoring Board	UE	Upper extremity
DVT	Deep vein thrombosis	ULN	Upper limit of normal
ECG/EKG	Electrocardiogram	UNK	Unknown
Hgb	Hemoglobin	VS	Vital signs
HTN	Hypertension	VEGF-C	Vascular Endothelial Growth Factor-C
ICG	Indocyanine Green	VM	Volume measurement
IDE	Investigational Device Exemption	VLNT	Vascularized lymph node transfer
IRB	Institutional Review Board	WBC	White blood cell
ISL	International Society of Lymphology	WHO	World Health Organization

1. OBJECTIVES

1.1. Primary Objective

- To determine whether the addition of the BioBridge scaffold to vascularized lymph node transfer will improve the outcome of surgical treatment of secondary arm lymphedema resulting from treatment of breast cancer. Primary endpoint is the post-surgical % change in excess limb volume, measured at 12 months following the surgical procedure. Intervention participants will be consented for safety follow-up for up to 36 months.

1.2. Secondary Objectives

- Change in measurement of dermal thickness, screening evaluations to Month 12, as measured by caliper skin-fold thickness.

1.3. Exploratory Objectives

- Change in L-Dex bioimpedance ratio, screening evaluations to Month 12
- Changes in dynamic lymphatic function by serial radionuclide LSG, performed at enrollment and 12 months after surgery.
- Changes in Lymphedema Quality of Life (LymQOL) score, screening evaluations to Month 12.
- Changes in quantifiable skin histopathology; cutaneous punch biopsies performed at enrollment and 12 months after surgery.

2. BACKGROUND

2.1 Study Disease

Lymphedema

Secondary (acquired) lymphedema is a serious, progressive and global disease that develops when the lymphatic system is physically compromised and unable to sufficiently transport interstitial fluid and macromolecules from affected region(s) of the body to the central circulation. In Western countries, it most commonly occurs as a delayed result of cancer treatment, most frequently in survivors of breast and gynecological cancers. In this highly prevalent condition, lymph node extirpation and the associated structural damage to the lymphatic vasculature leads to the inexorable accumulation of interstitial fluid, accompanied by regional compromise of immune function and, ultimately, irreversible structural changes of the affected tissues of the limb(s). [1] Lymphedema is a chronic, debilitating disease with profound functional and psychosocial implications. [2]

According to the American Cancer Society (ACS), in women there will be an estimated 252,710 new cases of invasive breast cancer, 63,410 of *in situ* breast cancer, and in men, 2,470 cases.[24] In 2017, approximately 40,610 women and 460 men are expected to die from breast cancer.

Development of upper extremity (UE) edema following axillary lymph node dissection for breast cancer treatment is the most common manifestation. Conservative estimates place the incidence of UE edema after axillary lymph node dissection at 15-26%. [3,5-7] Conservative axillary management (SLND) has resulted in reduced incidents of breast cancer related edema. [8-10]

To decrease the risk of recurrence for early-stage breast cancer patients with a positive SLN, radiotherapy is typically recommended. AMAROS trial results showed the rate of lymphedema in patients with early-stage breast cancer with positive SLN, was 15% at 1 year; 13.4% at 3 years; 10.3 at 5 years. Women who had ALND and axillary radiotherapy had the highest rates of lymphedema (25.6% at 1 year; 21% at 3 years; 20.8% at 5 years). [11]

2.2 Study Device/Procedure

The proposed study utilizes Fibralign's BioBridge™ Collagen Matrix, a sterile implantable biocompatible and biodegradable surgical mesh ribbon comprised of highly purified porcine collagen. The Class II device was cleared by CDRH Division of Surgical Devices on 8 January 2016 under 510(k) K151083. The device will be used for soft tissue surgical support at the time of vascularized lymph node transfer surgery (VLNT); the device will be used, specifically, for surgical support of the lymphatic component of the soft tissue.

We previously completed and published a large animal study that employed a well-studied and widely-published model. [43] The model replicates the pathogenesis of iatrogenic human secondary lymphedema (surgical removal of lymph nodes, radiation ablation). The study definitively demonstrated that, after BioBridge device implantation, the lymphatic tissue underwent repair during the period of experimental observation, with formation of new lymphatic vessels, restoration of function, and reduction in the tissue fluid accumulation that is associated with the untreated disease. Contrast-enhanced CT imaging and vital dye imaging confirmed that, at study conclusion, lymphatic vessels were fully functional within the irradiated tissue region, where there had been no demonstrable lymphatic vascular function before implantation of the BioBridge device. In the untreated control group, as expected, there was no observed improvement during this same period.

Post-mortem macroscopic analysis of the implantation area after in vivo intradermal injection of methylene blue demonstrated that, in BioBridge recipients, newly developed lymphatic collectors could be identified; the development of new collectors was corroborated by post-treatment CT imaging. During the course of the study, 120 BioBridge devices were implanted, with no identified complications during the 3 months after implantation or through histological analysis.

This preclinical study demonstrates the efficacy and safety of BioBridge for the enhancement of lymphatic repair and revascularization following the development of post- surgical acquired lymphedema in an in vivo model that simulates the human condition of acquired lymphedema of the limb.

Transplantation of healthy tissues to replace or re-route damaged lymphatic vessels, in which only arterial and venous vessels are reconnected, but in which lymphatic vessels cannot be re-anastomosed, has shown improvements in limb edema, with evidence of lymphatic re-routing and clinical evidence of spontaneous lymphatic regeneration. Although lymph node transfer has been shown to provide some benefit in human lymphedema patients, autologous lymph nodes incorporate into existing lymphatic vasculature at a relatively low frequency, thus compromising the outcome, inasmuch as connection with lymphatic vessels is required for the maintenance and function of the transplanted lymph nodes. It is our belief that the addition of the Fibralign BioBridge will address a key weakness of this procedure, by bridging across damaged tissue and providing the mechanical support needed to effectuate repair, lymphatic anastomosis and restoration of lymphatic function. [3]

Fibralign's BioBridge Collagen Matrix consists solely of highly purified collagen with the immunogenic telopeptides removed. This starting material is already used in commercial implant devices and sterilized after fabrication and packaging. Extensive GLP biocompatibility testing has already been successfully completed in support of the 510(k) clearance and provided earlier to the FDA, including implantation studies that show BioBridge naturally and gradually incorporates into the host tissue within 6 to 9 months after implantation, ensuring a safe application in lymphedema treatment.

One subject has been treated under this protocol (IRB-37161) prior to December 2019 revision cycle, which was implemented at the request of the NIH funding source. The single previously-enrolled IRB-37161 subject underwent VLNT with BioBridge placement per protocol. Due to the significant change in this study, this patient will not be included in the final analysis.

Since 2018, the BioBridge Collagen Matrix surgical mesh ribbon has been implanted as medical care in a number of lymphedema patients (personal communication, Dung Nguyen, MD.[49] Summary information is available at this time on 23 patients. 20 patients had secondary lymphedema post-cancer treatment, and a few had lymphangiomatosis or primary lymphedema. Of the cancer patients; 13 had breast cancer and 6 had gynecological cancer or melanoma. All had undergone lymphatic surgery prior to BioBridge placement. 14 patients had undergone vascularized lymph node transfer (VLNT); 5 underwent LVA; and 3 received combined VLNT with lymphaticovenous anastomosis (LVA). The time interval between the lymphatic surgery and BioBridge placement ranged from 1 month to 28 months. One patient, with secondary lymphedema post-cancer treatment for Hodgkins's lymphoma, had the BioBridge implanted with their lymphatic surgery (liposuction and VLNT). [50]

Based on available data, 15 of 23 patients, covering pre-surgery assessments, pre-BioBridge placement surgery, and post-BioBridge placement data, all Dr Nguyen's patients showed improvement of excess volume after their initial lymphatic surgery, ranging from 2% to 33% improvement in volume. Of these, 13 of 15 showed improvement of excess volume after BioBridge placement that ranged from 1% to 29%. In the remaining 2 patients, there was a

median increase of volume of 6.5% in limb volume. The truncated cone formula, as described by Dr Håkan Brorson, was used to calculate excess volume. [42]

For clinicaltrials.gov compliance

IRB-37161 information was entered into the ClinicalTrials.gov record in 2016 as NCT02734979) and will be updated after IRB approvals, as appropriate.

2.3 Rationale

Currently, there is a paucity of effective treatment options for lymphedema of the limb(s). These include compression garments, intermittent compression devices, complete decongestive physiotherapy (CDPT), skin care, and exercise. The current standard of care is a combination of these approaches. CDPT, which combines manual lymph drainage, multilayer short-stretch bandaging, exercise and skin care was recommended by a consensus panel of experts as the standard of care. [12-18] Utilizing this technique have resulted in reduction in excess limb volumes in the range of 50%. [4,19-21] Compete resolution is rarely achieved, is costly, and is time and labor intensive. [22] While modestly effective, the underlying cause is not addressed.

In a completed large animal model (porcine) of human lymphedema, of the utilization of the Fibralign Biobridge, had the capacity to guide and augment lymphatic repair, and thereby reverse the pathologic burden of lymphedema on the tissues of the affected limb(s). [43] The BioBridge is an implantable, interventional device comprised of a thread-like, multi-lumen collagen scaffold. Biobridge's highly aligned nanofibrils encourage the endothelial cell attachment, alignment and migration that are prerequisite to new vessel formation. [44,45] In our completed preclinical investigation, BioBridge implantation successfully induced lymphatic regeneration and ameliorated the pathology in a porcine lymphedema model (funded through a USAMRAA CDMRP Breast Cancer Research SBIR Phase II grant). The results of these investigations have been presented at several national and international biomedical congresses and the manuscript is under peer review for publication. On the basis of the completed preclinical investigations, the FDA has granted a 510(k) for this device. IDE was presented and approved by the FDA. An amended IDE application, for randomized study, will be presented to the FDA for their approval.

On the strength of our preclinical findings, we propose a prospective clinical investigation of the impact of BioBridge as an adjunct to VLNT. VLNT is an increasingly practiced surgical intervention for limb lymphedema, despite the fact that the documented success rate of the current procedure is not optimal. Of published studies reporting limb volume outcomes after VLNT, volume changes range from an increase of 13% to a decrease of 64% (from presurgical volume). [34] Animal studies suggest that surgical failure may be due to inadequate lymphatic vascular engraftment of the transplanted node. [48] VLNT with placement of the BioBridge scaffold is intended to provide lymphatic soft tissue support that is needed for effective lymphatic revascularization of the transplanted node. It is hoped to demonstrate that the BioBridge improves upon the less-than-optimal success rate of the currently practiced surgery

by facilitating the lymphatic connections to the transplanted node that are crucial to its viability and function. [34] The proposed study will evaluate the clinical efficacy of adjunctive BioBridge use in VLNT. We hypothesize that BioBridge will have the capacity to substantially improve the efficacy of VLNT, providing the stable and optimally predictable outcomes that are currently lacking for the unaided procedure. We have therefore designed a novel approach to treatment that directly addresses a component of the underlying cause and may be more effective as a treatment strategy.

2.4 Study Design

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance

- Primary purpose of the protocol:
  - **Treatment**: protocol designed to evaluate one or more interventions for treating a disease, syndrome or condition
- Interventional model:
  - **Parallel**: one of two groups in parallel for duration of study.
- State the number of intervention arms
  - **Two arms**
- State whether the study will be masked (at least one party is unaware of the treatment)
  - **Open**: no masking is used
- State whether the study is randomized.
  - **Randomized**
- Primary outcome or outcome that the protocol is designed to evaluate:
  - **Efficacy**

2.5 Correlative Studies Background

We previously completed and published a large animal study that employed a well-studied and widely-published large animal model of lymphedema. [43] The porcine hindlimb model replicates the pathogenesis of iatrogenic human secondary lymphedema (surgical removal of lymph nodes, radiation ablation). The study definitively demonstrated that, after BioBridge device implantation, the lymphatic tissue exhibited successful repair during the period of experimental observation, with formation of new lymphatic vessels, restoration of function, and reduction in the tissue fluid accumulation that is associated with the untreated disease. Contrast-enhanced CT imaging and vital dye imaging confirmed that, at study conclusion, lymphatic vessels were fully functional within the irradiated tissue region, where there had been no demonstrable lymphatic vascular function before implantation of the BioBridge device. In the untreated control group, as expected, there was no observed improvement during this same period.

Post-mortem macroscopic analysis of the implantation area after in vivo intradermal injection of methylene blue demonstrated that, in BioBridge recipients, newly developed lymphatic collectors could be identified; the development of new collectors was corroborated by post-treatment CT imaging. During the course of the study, 120 BioBridge devices were implanted, with no identified complications during the 3 months after implantation or through histological analysis.

The details of the model are described here. A porcine model for secondary lymphedema in the minipig (Yucatan breed) was developed through meticulous resection of the lymphatic system of one hind limb and subsequent radiotherapy. This procedure led to chronic edema in 44% of the animals at the 3-month time point, as defined by a bioimpedance spectroscopy (BIS) ratio greater or equal to 1.05. The relative incidence of lymphedema in the porcine model and post-operative wound healing problems were comparable to those observed in humans after similar procedures. [1] Morphological features of chronic lymphedema in the minipigs were confirmed by computed tomography (CT) and MRI; the number of CT-identified lymphatic collectors correlated with the lymphatic dysfunction as determined by BIS. [28] Using BIS combined with CT imaging to identify active lymphedema, 50% of the animals in the untreated control group had an initial diagnosis of lymphedema at the 3-month time point, and in all these animals, the lymphedema persisted to the 6-month observation point.

Lymphedema treatment with BioBridge

Three months after lymph node resection and radiotherapy, animal subjects underwent surgical intervention, with implantation of BioBridge scaffolds spanning the area depleted of lymphatics. The treatment options included implantation of: 1) scaffold only; 2) transplantation of autologous lymph node fragments (LNF) supplemented with VEGF-C-enriched scaffold; or 3) a VEGF-C treated scaffold. Untreated control subjects received no treatment.

All animals with pre-treatment lymphedema in Group 2 (BioBridge with LNF) experienced resolution of the lymphedema, and those in Group 1 (BioBridge only) showed improvement after 3 months as demonstrated by reduction of bioimpedance spectroscopy ratios and increase in CT-detectable lymphatic collectors vs pre-treatment levels. In both of these treatment groups, animals without lymphedema prior to surgical treatment did not subsequently develop it. In contrast, in Group 3 (BioBridge with VEGF-C) and the control group, the majority of the animals had lymphedema either persist or manifest after the treatment started.

Post-mortem macroscopic analysis in animals with lymphedema resolved or improved showed that new lymphatic collectors in the area of prior surgery and radiation could be seen through the skin after prior peripheral injection of blue dye and were confirmed by CT-imaging to be in the vicinity of the implanted collagen threads. Macroscopically, newly formed collectors had no abnormal architecture. In contrast, in control animals from Group 3, post-mortem examination disclosed visible lympho-vascular conglomerates in the vicinity of the distal end of the implant.

Standard histological analysis demonstrated integration of the nanofibrillar collagen scaffold into the irradiated tissues.

Confocal immunohistochemistry revealed a higher number of lymphatic collectors in the proximity of the implanted collagen scaffold, when compared to the surrounding irradiated tissue or to untreated irradiated tissue. The number of blood vessels was also increased in this area, however the balance between the lymphatic and blood vessels in the vicinity of the scaffold was shifted toward lymphatics, as shown by the increase in the lymphatic fraction of the total microvascular density (lymphatic + blood) when compared to untreated irradiated tissue.

In summary, this preclinical large animal study demonstrated the efficacy and safety of BioBridge for the enhancement of lymphatic repair and revascularization following the development of post- surgical acquired lymphedema in an in vivo model that simulates the human condition of acquired lymphedema of the limb.

In subjects with lymphedema, implantation of BioBridge 1) normalized or reduced the extracellular liquid volume and 2) increased the number of functional lymphatic collectors, accompanied by 3) BioBridge integration into irradiated tissue and 4) formation of new microvasculature with increased lymphatic fraction in the proximity of the scaffold. Implantation of BioBridge supported by lymph node fragment transfer had the most beneficial effect, with all animals showing no signs of lymphedema at the experimental conclusion, while BioBridge supplemented with VEGF-C, a surgical control in which the directional signaling was aborted, did not improve lymphedema condition. Careful consideration of current methods to treat lymphedema in established clinical practice suggests that supplementing the vascularized lymph node transfer procedure with BioBridge will increase the magnitude of the clinical response in lymphedema patients.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

The subject must be a breast cancer survivor, at least 3 years beyond completion of cancer therapy, free of clinical disease, and eligible for surgical intervention. Participants who are not able to safely undergo general anesthesia and/or perioperative care for VLNT are excluded (See Exclusion Criteria 3,2,2).

3.1.1 Ages 18 to 75 years (inclusive)

3.1.2 Eastern Cooperative Oncology Group (ECOG) Performance Status 0 to 2

3.1.3 Life expectancy > 2 years

3.1.4 Acquired (secondary) upper limb lymphedema secondary to breast cancer treatment

3.1.5 The participant must be eligible for surgical intervention

3.1.6 Swelling of 1 limb that is not completely reversed by elevation or compression

3.1.7 Stage II or greater lymphedema at screening, based on the International Society of Lymphology (ISL) staging system

3.1.8 Participants must have no evidence of disease (NED), have completed breast cancer therapy 3 years prior to enrollment; use of endocrine therapy is allowed.

3.1.9 Completion of a full course of complete decongestive therapy (CDT), according to ISL guidelines at least 8 weeks prior to screening, including use of compression garments for at least 8 weeks without change in regimen

3.1.10 Willingness to maintain a stable regimen of self-care, with consistent use of compression garments from screening through the entire study duration (through the safety follow-up visit). Self-bandaging, use of nighttime compression garments, and intermittent pneumatic compression devices are allowed, but the procedures and regimens are expected to remain consistent from screening though the entire study duration.

3.1.11 Consistent use of an appropriately-sized compression garment for daytime use

3.1.12 ,Two consecutive measurements of limb volume (LV) in the affected limb, taken at least 1 day apart during the screening period, must be within 10% of each other. A maximum of 3 measurements can be taken. Affected limb volume ratio must be ≥ 20% (compared to unaffected limb); volume measurements will be performed and volume ratio will be calculated at S1 and S2 visit.

3.1.13 Evidence of abnormal bioimpedance ratio, if feasible, based upon unilateral disease: L-Dex > 10 units; bioimpedance performed at S1 and S2

3.1.14 Willingness and ability to comply with all study procedures, including measurement of skin thickness using skin calipers

3.1.15 Willingness and ability to understand, and to sign a written informed consent form document

3.2 Exclusion Criteria

3.2.1 Edema arising from increased capillary filtration will be excluded (venous incompetence).

3.2.2 Inability to safely undergo general anesthesia and/or perioperative care related to vascularized lymph node transfer

3.2.3 Concurrent participation in a clinical trial of any other investigational drug or therapy, regardless of indication, within 1 month before screening or 5 times the drug's half-life, whichever is longer

3.2.4 Recent initiation of (≤ 8 weeks), or intention to initiate, CDPT or maintenance physiotherapy for lymphedema at any time during the duration of the study

3.2.5 Other medical condition that could lead to acute limb edema, such as (but not limited to) acute venous thrombosis

3.2.6 Other medical condition that could result in symptoms overlapping those of lymphedema in the affected limb (eg, pain, swelling, decreased range of motion)

3.2.7 History of clotting disorder (hypercoagulable state)

3.2.8 Chronic (persistent) infection in the affected limb

3.2.9 Any other infection (unrelated to lymphedema) within 1 month prior to screening

3.2.10 Currently receiving chemotherapy or radiation therapy

3.2.11 Current evidence of active malignancy, or a history of malignancy within the past 3 years (except for non-melanoma skin cancer or cervical cancer in situ treated with curative intent). If the participant has undergone cancer treatment, this must have been completed > 3 years prior to enrollment.

3.2.12 Current evidence of any high risk for recurrence of breast cancer [eg, Stage III or IV; estrogen receptor (ER) / progesterone receptor (PR) / HER-2 negative (ie, "triple-negative") cancer; locally-advanced breast cancer; inflammatory breast cancer; > 3 positive axillary lymph nodes; extracapsular nodal extension; invasive micropapillary breast carcinoma; or if performed, genetic testing, eg, BRCA1; BRCA2; Oncotype DX (high-risk recurrence score); or Mammaprint (poor risk signature) indicating a high risk for breast cancer recurrence

3.2.13 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

3.2.14 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

3.2.15 Absolute neutrophil count < 1500 mm^3 at screening

3.2.16 Hemoglobin concentration < 9 g/dL at screening

3.2.17 Known sensitivity to porcine products

3.2.18 Hypersensitivity to iodine

3.2.19 Pregnancy or nursing

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

3.2.21 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

We will use a blocked randomization design. Approximately 60 subjects will be randomized in a 4:1 ratio to the intervention arm (VLNT + BioBridge): control arm (VLNT alone). Randomization will be done within blocks (of size 5) so that the balance between treatments is preserved throughout the trial, where A = VLNT/Biobridge and B = VLNT alone.

The order in which these blocks will be assigned to the enrolled subjects will be prospectively, independently determined through a random number generator function.

3.5 Study Timeline

Primary Completion:

- The study will reach primary completion 48 months from the time the study opens to accrual.

Study Completion:

- The study will reach study completion 72 months from the time the study opens to accrual.

4. TREATMENT PLAN

Pre-op procedure:

- LSG baseline (it may be the same as one performed for research purposes)
- Venous duplex to evaluate the superficial and deep venous systems for clots and valvular insufficiency
- ICG fluorescence lymphatic mapping to assess the superficial lymphatic system
- Labs: CBC, CMP, PT/PTT, EKG, CXR
- Research samples: blood and skin samples; optional for control group
- Baseline photograph of limbs

Post-op:

- LSG at 12 months (it may be the same as one performed for research purposes)
- ICG fluorescence lymphatic mapping at 12 months

- Research samples: blood and skin samples; skin biopsies are optional for control group
- Photograph at 12 months

Time of procedure:

Operating time ~6 hours, additional 45 min for BioBridge placement; anesthesia ~10 to 12 hours, positioning, IV and arterial line placement, OR, etc (time is variable).

Description of procedure:

On the day of surgery, the patient will be sent to radiology for injection of Technecium-99 in the second web space of bilateral feet or contralateral arm in preparation for intraoperative lymph node transfer. Patient will be marked in the preoperative area. Patient will then be taken to the operating room, placed on the operating room table in the supine position with both arms abducted. Perioperative antibiotic will be given on-call to the OR. After general endotracheal anesthesia is established, the entire anterior chest, abdomen, proximal thighs and arm(s) will be prepped and draped in the usual sterile fashion. A surgical pause is taken to identify the patient and the procedure(s) to be performed.

Attention will be turned to the left or right groin lymph node region or right or left neck for supraclavicular lymph node region. A Doppler is used to confirm the location of the lymph node pedicles. Next, reverse lymphatic mapping is used as an additional method to confirm the location of the lymph nodes. Technecium-99 will be injected preoperatively into the foot or hand (depending on donor site). A gamma probe is used to localize the sentinel lymph node draining the extremity, which will be avoided during flap harvest. The highest gamma probe count is noted and the donor site is deemed suitable only if the gamma probe count for the planned lymph node donor site is <10% of the previously noted maximum. Indocyanine green (ICG) 0.2 mL is injected intraoperatively into several sites along the periphery of the donor flap (lower abdomen or supraclavicular) for lymphatic mapping. Fluorescence angiography is used to visualize the lymph nodes draining the lower abdominal wall or supraclavicular region.

Groin donor flap:

The lymph nodes draining the lower abdomen are visualized and correlated with the SCIA-based lymph nodes. An incision is made into the lower (left or right) abdomen and the skin flaps are elevated just above the Scarpa fascia. At the superior edge of the flap, the SCIV and SIEV are both identified and divided, with the knowledge that most of the target lymph nodes will lay between these vessels. The flap will be elevated lateral to medial just above the sartorius fascia. The SCIA branch to the Sartorius muscle is divided, and the SCIA and branches of the SCIV are mobilized to their origin and are harvested for transfer.

The groin wound is irrigated with an antibiotic solution and hemostasis is achieved. The inguinal fascia, if needed, may be reinforced with an onlay polypropylene mesh, secured with interrupted and running 2-0 Prolene sutures. A Blake drain will be placed to drain the groin

wound. The wound is closed with interrupted 2-0 Vicryl sutures for the Scarpa, interrupted 3-0 Vicryl sutures for the dermis and 4-0 Monocryl running subcuticular stitch.

Supraclavicular donor flap:

A transverse incision, 1 cm above the clavicle in the posterior neck triangle, will be made. The superior skin flap will be elevated in the subplatysmal plane using a bovie. Dissection will proceed medial to lateral to include all the intervening subcutaneous tissues and lymph nodes in the posterior triangle below the omohyoid muscle. The transverse cervical artery and vein will be identified. Once the flap is islandized on its pedicle, a handheld Doppler is used to confirm perfusion in the lymph node flap.

The pedicle to flap will be ligated and clipped. The donor site is irrigated and hemostasis confirmed. A Blake drain will be placed in the wound, and secured to the skin with Nylon suture. The platysma is reapproximated with 3-0 Vicryl sutures, and the wound will be closed with interrupted 3-0 Vicryl sutures for the dermis and 4-0 Monocryl running subcuticular stitch. The incision is cleaned and dressed with dermabond, Telfa and a sterile pressure dressing.

Next, the recipient vessels are dissected in the upper arm. A curvilinear incision will be made in the medial posterior aspect of the arm above the elbow crease. The skin flaps are elevated off the underlying structures using a bovie to create a pocket for the fascial-lymph node flap. Small superficial veins are identified and dissected free. The basilic vein and medial cutaneous nerve of the arm are identified and preserved. The superior or inferior recurrent ulnar artery is identified, and dissected distally to gain pedicle length. The lymph node dermal fat flap is then harvested; the SCIA and SCIVs are anastomosed to the distal end of the superior or inferior recurrent ulnar artery and its vena commitantes using interrupted 10-0 or 11-0 nylon sutures for the arterial anastomosis and venous couplers.

SutureEase device will be used to tunnel five 15 cm long Fibralign BioBridge Collagen Matrix individually in the subcutaneous tissue from the lymph node flap down to the mid forearm. 3 additional 15 cm long BioBridge Collagen Matrix are similarly tunneled proximally pass the shoulder toward the neck and two 15 cm long BioBridge Collagen Matrix are tunneled to the axilla. Prior to implantation, the BioBridge is moistened in saline, to facilitate gliding in tissue. Once this is completed, the skin flaps are redrapped over the fascial-lymph node flap and closed with interrupted 3-0 Vicryl sutures for the dermis and 4-0 Monocryl running subcuticular stitch for the skin. The total length of time spent on placement of the Biobridges is about 45 minutes.

At the end of the procedure, Doppler signal from the flap, correct sponge and needle counts will be verified. Minimal blood loss is expected. The patient will be awakened; extubated; and transferred to the recovery room.

Standard PACU recovery: 1 to 1.5 hrs.

Hospitalization: 2 to 3 days, managed postoperative in accordance to the institution standardized free flap protocol.

Post-op instructions (specific to this procedure-VLNT): patients are instructed to keep their surgical arm elevated x 3 weeks at level of heart or above. Otherwise, patient may ambulate, eat and resume normal light activities that does not require use of treated arm. Patients will be instructed to not wear compression garments for 3 weeks post-operative.

Participants will be given consent form and contact information to take home for their consideration. Samples (skin punch biopsy and blood) may be retained for future research use if patient has given consent (in ICF). Pre-treatment and post-treatment photographs will be taken.

Study Visits

There are 3 screening visits.

S1: after obtaining written consent, medical history, symptoms, current medications, and allergies will be reviewed, height/weight/VS, physical exam by PI will be conducted, limb measurements performed (volume, BIS, caliper), and LymQoL survey completed.

S2: VS, weight, medication and allergy review, measurements; if measurements are consistent, will proceed to skin punch biopsy (optional for control group); research labs will be drawn. No compression garment wear x 10 days.

S3: VS, weight, medication and allergy review, measurements, stitch removal, LSG. Patients resume wearing compression; after 3 weeks, eligible to proceed with surgery.

VLNT, 2 groups; one with BioBridge scaffold (intervention group), one VLNT only (control group), will occur between S3 and T1 visits.

T1 (3 months following surgery): physical exam, measurements, LymQoL survey, medication and symptom/AE review, VS, weight.

T2 (6 months following surgery): physical exam, measurements, LymQoL survey, medication and symptom/AE review, VS, weight

T3 (9 months following surgery): physical exam, measurements, LymQoL survey, medication and symptom/AE review, VS, weight.

T4 (12 months following surgery): physical exam, measurements, LymQoL survey, medication and symptom/AE review, VS, weight, skin punch biopsy (optional for control group), research labs. No compression garment wear x 10 days.

T5 (10 days after T4 visit): measurements, LymQoL survey, medication and symptom/AE review, VS, weight, stitch removal, LSG

T6 (2 years following surgery, BioBridge participants/intervention group): physical exam, medication and symptom/AE review, VS, weight.

T7 (3 years following surgery, BioBridge participants/intervention group): physical exam, medication and symptom/AE review, VS, weight.

4.1 General Concomitant Medication and Supportive Care Guidelines

For VLNT, the following is routinely used pre-operatively (standard of care, SOC):

Chlorhexidine gluconate scrub.

Anesthesia risks (anesthesia and intubation) (SOC for VLNT placement; research for BioBridge placement):

Intubation injury; aspiration; anesthesia awareness; difficulty breathing after surgery; malignant hyperthermia; reaction to anesthesia drugs; nausea; vomiting; dry mouth; sore throat; shivering; sleepiness; confusion; hoarseness.

For VLNT, the following medications are routinely used intra-operatively (SOC for VLNT placement; research for BioBridge placement):

Midazolam; fentanyl; lidocaine; propofol; rocuronium; dexamethasone; ketamine; hydromorphone; acetaminophen; ondansetron; antibiotic (usually cephalexin unless allergic); scopolamine; magnesium; IV fluids (saline, lactated ringers).

Arterial line risks (SOC): Bleeding; hematoma; thrombosis; infection.
Rare risks: permanent arterial occlusion; pseudoaneurysm, sepsis.

Surgical risks (SOC for VLNT placement; research for BioBridge placement):

Bleeding; deep vein thrombosis (DVT); delayed healing; death; infection; injury during surgery; medical complications (pneumonia; MI; CVA; itching; scarring; numbness; neurovascular injury; tingling around surgical site(s); pain; persistent edema; seroma; flaps loss; donor site lymphedema; bruising; and need for reoperation.

For VLNT, the following medications are routinely used post-operatively (SOC):

Oxycodone; aspirin; ondansetron; Bacitracin zinc ointment; Lovenox; hydromorphone; heparin infusion

Lymphoscintigraphy (SOC for VLNT placement; research for BioBridge placement):

LSG will be performed for both research purposes and standard of care (1-2 scans pre-surgery and 1 to 2 scans 12 months post-surgery).

Lymphoscintigraphy requires four injections of 250uCi Sulfur Colloid, is performed at baseline and 12 months after surgery, and involves exposure to radiation. The LSG for research are not necessary for medical care. The additional amount of radiation exposure is about 7 mSv, which is approximately equal to 14% of the limit that radiation workers (for example, a hospital X-ray

technician) are allowed to receive in 1 year. This amount of radiation involves minimal risk and is necessary to obtain the research information desired.

Because the doses of radiotracer administered are small, diagnostic nuclear medicine procedures result in relatively low radiation exposure to the patient and the radiation risk is low. Nuclear medicine diagnostic procedures have been used for more than 5 decades, and there are no known long-term adverse effects from such low-dose exposure. Allergic reactions to radiopharmaceuticals may occur but are extremely rare and are usually mild. Injection of the radiotracer can be painful and may cause redness which should rapidly resolve.

Technetium 99m sulfur colloid is a nuclear imaging agent. The most frequently reported adverse reactions include rash; allergic reaction; hives; allergic shock; and low blood pressure. Less frequently reported adverse reactions are fatal cardiopulmonary arrest; seizures; shortness of breath; wheezing; abdominal pain; flushing; nausea; vomiting; itching; fever; chills; perspiration; numbness; and dizziness. Local injection site reactions; including burning; blanching; redness; swelling; and scarring, have also been reported.

ICG fluorescence lymphatic mapping (SOC, at baseline and 12 months post-surgery)

ICG (indocyanine green) is a water-soluble tricarbocyanine dye, has a short plasma half-life of 3 to 5 minutes in humans, is excreted exclusively by the liver into the bile, and is not associated with risk for nephrotoxicity. ICG contains sodium iodide and is contraindicated in patients with iodine hypersensitivity. Anaphylaxis or other allergic reactions may occur.

Skin punch biopsy (research):

Skin biopsies of affected limb will be performed at baseline and at 12 months following surgery (optional for control group). Potential side effects include pain, infection and bleeding. Participants will receive 48 hours of prophylactic oral antibiotic to mitigate the potential for infection. Bleeding should be minimal as only a 6 mm biopsy lesion will be utilized. Each incision is closed with one suture.

For biopsy, xylocaine with epinephrine:

Xylocaine with epinephrine is used as a local anesthetic. Potential risks include: feeling anxious; shaky; dizzy; restless; or depressed; drowsiness; vomiting; ringing in your ears; blurred vision; confusion; twitching; seizure (convulsions); fast heart rate; rapid breathing; feeling hot or cold; weak or shallow breathing; slow heart rate; weak pulse; or feeling like you might pass out. Less serious side effects include: mild bruising; redness; itching; or swelling where the medication was injected; mild dizziness; nausea; numbness in places where the medicine is accidentally applied.

For biopsy, cephalexin (unless allergic to this antibiotic):

Cephalexin is an antibiotic. Possible adverse effects include the following:

- Central nervous system: Agitation, confusion, dizziness, fatigue, hallucinations, headache
- Dermatologic: allergic swelling, severe rash, Stevens-Johnson syndrome, toxic epidermal necrolysis, hives
- Gastrointestinal: Abdominal pain, diarrhea, stomach burning, gastritis, nausea , colitis, vomiting
- Genitourinary: Genital itching, genital thrush, vaginitis, vaginal discharge
- Hematologic: elevated white blood cell counts
- Hepatic: abnormal liver function, jaundice , transient hepatitis
- Neuromuscular & skeletal: joint aching and swelling, joint disorder
- Renal: kidney inflammation
- Miscellaneous: Allergic reactions

Phlebotomy (SOC and research):

Risks of blood samples being drawn include: pain; bruising; bleeding; inflammation; infection; temporary redness of the skin where the injection is given; and light headedness. Care will be taken to avoid these difficulties.

Bioimpedance spectroscopy (BIS) (research):

Placement of and/or removing the pads may cause skin irritation; BIS analysis is painless.

Calipers (research):

The calipers may cause some discomfort due to the pinching of the skin.

Stanford Experience

Dr Nguyen has performed more than 100 cases of VLNT over the last 5 years. The average volume reduction is 55 to 65%. There has not been any flap loss. There is < 5% post-operative infection; < 5% wound dehiscence; 2% of seroma; 2% of hematoma; 1% post-operative deep vein thrombosis (DVT). There have had no major complications. To date, there has been no incidence of donor site lymphedema. In general, this is a well-tolerated operation. The positive outcome is, in part, dependent upon patient compliance with postoperative instructions.

4.2 Criteria for Removal from Study

It will be documented whether or not each subject completed the clinical study. Subjects who discontinue from the study early will be asked to return for a final study visit within the 4 weeks following the decision to withdraw. If a subject withdraws, all efforts will be made to complete

and report the observations, particularly the follow-up examinations, as thoroughly as possible. A sincere effort will be made to contact subject either by telephone, letter, email, to determine the reason why they failed to return for the necessary visits or withdrew from study, and reason(s) will be documented.

Reasons that a subject may discontinue participation in a clinical study may include the following:

- Intercurrent illness that prevents continuation in study
- Potential health hazard to patients, as indicated by the incidence or severity of AEs
- The subject may choose to withdraw from the study at any time for any reason
- General or specific changes in the patient's condition that render the subject unacceptable for further treatment in the judgment of the investigator
- Severe non-compliance to protocol as judged by the investigator
- Death
- Closure of study

4.3 Alternatives

Alternatives to participation in this trial include enrollment in VLNT without the addition of the BioBridge scaffold or standard of care treatments for lymphedema. These include, but are not limited to, compression sleeves or compression devices, decongestive therapy, intermittent pneumatic compression devices. In general, there are 4 types of surgical intervention for lymphedema, as follows. [35,36,46-48]

Lymphatic Microsurgical Preventive Healing Approach (LYMPHA), aimed at lymphedema prevention, was developed at the University of Genoa. [37,38] At the time of axillary lymph node dissection, a lymphaticovenous anastomosis (LVA) is performed to restore lymphatic flow. While a comparatively new procedure, for patients undergoing ALND, results have been very promising. [39] However, since it is aimed at lymphedema prevention, LYMPHA is not an appropriate alternative for this participant population, since inclusion into this trial requires a diagnosis of lymphedema.

Excisional surgery, suction-assisted protein lipectomy (SAPL), pioneered by Dr Håkan Brorson, is performed for chronic non-pitting lymphedema. SAPL is highly effective at removing the excess solid tissue (hypertrophied adipose tissue) but does not restore lymphatic function. Dr Nguyen, who trained with Dr Brorson, frequently performs this procedure. Post-operatively, the continuous use (24 hours/day, 7 days/week) of custom compression garments is required. These may need to be frequently replaced in the 1st year after the procedure. Maintenance of reduction is dependent on patient compliance with continuous compression garment use. [40] SAPL is also not an appropriate alternative, since its aim is removal of excess tissue and not the restoration of lymphatic function.

Microvascular reconstruction with LVA is also performed by Dr Nguyen. A large study, by Chang, *et al*, noted that LVA, is most effective in early stage lymphedema of the upper extremity. Early volume reduction may be seen in the first postoperative week but maintenance of response is unknown due to short follow-up. [41] Additionally, LVA may not enhance the regional immune impairment that accompanies lymphedema.

Tissue transfer, ie, vascularized lymph node transfer (VLNT), when successful, is intended to optimally restore both lymphatic circulatory and immune function. The BioBridge scaffold has been designed, and tested in preclinical animal models, to optimize the surgical outcome specifically in vascularized lymph node transfer. It is this surgical intervention that is under specific investigation in this protocol.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent/Device/Procedure

Complete information about the investigational device can be found in the Investigator's Brochure and IDE document.

The BioBridge Collagen Matrix is a sterile implantable biocompatible and biodegradable surgical mesh ribbon comprised of highly purified porcine collagen that is designed to provide mechanical support and repair for weaknesses and deficiencies in soft tissue. The device was cleared by CDRH Division of Surgical Devices on 8 January 2016 under 510(k) K151083 for use as a collagen-derived surgical mesh for plastic and reconstructive surgery. For the purposes of this investigation, the commercially-available device will not be altered or modified in any way.

The collapsed ribbon structure of BioBridge is comprised of a single sheet of cross-linked collagen collapsed into multiple folds forming a thin membrane with aligned collagen fibrils in the same lengthwise direction. This scaffold structure provides mechanical properties that contribute to strong tensile strength, which provides support to weaknesses and deficiencies in soft tissue and aids in bridging a connection between two healthy soft tissues.

BioBridge is fabricated using a proprietary manufacturing process that produces a narrow and very thin ribbon-like membrane comprised of aligned collagen fibrils, creating a multi- luminal structure that can be sutured, and provides the desired mechanical properties for support of soft tissue repair. This approach presents the opportunity to enable the use of highly purified collagen, in a defined structure that has mechanical properties similar to those of the predicates, but without undesirable telopeptides found in collagen matrices derived from animal dermis. The smaller ribbon-like form factor also gives the surgeon greater flexibility for minimally invasive delivery and to tailor their procedures to better address a specific patient need, where one or more BioBridge devices can be implanted depending on the surgeon's discretion.

Fibralign sources the highly purified Type I porcine-derived collagen from a FDA registered and ISO-qualified supplier. Fibralign employs a proprietary manufacturing process that takes highly purified Type I porcine-derived collagen and produces narrow, ribbon-like membranes

comprised of highly aligned collagen fibrils that mimic desired mechanical properties found in natural extracellular matrices. A chemical crosslinking agent, known as EDC [N-ethyl-N'-(3 -dimethylaminopropyl)carbodiimide], is used during the manufacturing process to promote the crosslinking of the collagen but the crosslinker itself is not added to nor bound to the collagen matrix. The crosslinker residuals are water soluble and are removed by product rinsing at the end of the production process. The final product is packaged into individual storage tubes and then sealed in a multi-pack and terminally sterilized.

BioBridge is fabricated using a proprietary manufacturing process that collapses a single sheet layer along the width direction to form a scaffold, comprised of collagen fibrils (see Figure 1).

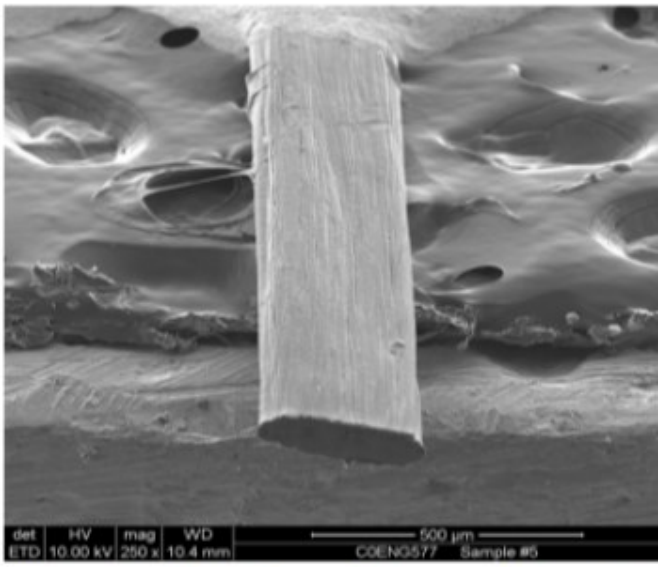


Figure 1. BioBridge (250x magnification)

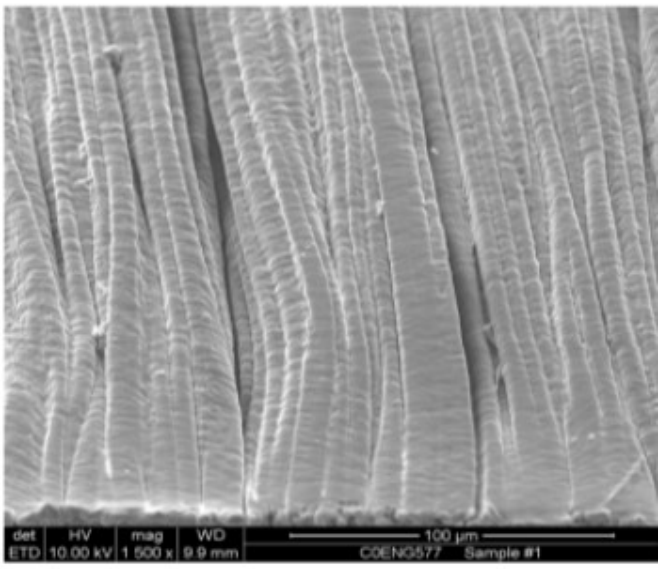


Figure 1. BioBridge Collagen Matrix.

BioBridge has a thickness in a range of 0.10 mm to 1.0 mm, and the device is available in lengths over 1500 mm.

Extensive safety and performance testing has been completed for BioBridge in support of the 510(k) clearance, including biocompatibility and degradation studies, as well as full material characterization of the collagen content and manufacturing reagents used in fabricating BioBridge (provided as part of the 510(k) submission). Significant efforts have been made in sourcing materials, product design and device manufacturing to minimize potential risks associated with the device.

These include:

1) BioBridge is made of highly-purified, pepsin-treated porcine Collagen Type I. The material is sourced from Datum Dental, which is referenced in the FDA 510(k). The same material is used in ColBar Ossix and ColBar Ossix Plus which have 510(k) clearance and CE mark and been used in over 350,000 implant procedures worldwide. Porcine-derived collagen matrix products have a long history of safe use as a surgical support device where the physical construct of the implant can provide a bridging material to support a desired surgical outcome. This same type of collagen is commonly used in very sensitive applications like dura layers involving the spinal cord and brain.
2) BioBridge is manufactured from this highly purified collagen in a GMP cleanroom facility under Class-100 hoods with upmost care and then e-beam sterilized after packaging. This has been supported by shelf life age testing and sterilization validation.
3) The BioBridge matrix is a very narrow ribbon, a thread-like structure that has a similar form factor to suture. In practice, the proposed treatment involves a very small amount of bulk material (10 mg/device, with 4 to 10 devices/patient) being implanted subcutaneously.
4) BioBridge has been shown to be biologically more inert than catgut suture, which is being commonly used in the surgical procedures proposed in the study. This was demonstrated in four GLP implantation studies that were performed in support of the 510(k) application. The subcutaneous implantation studies (2, 13 and 26 week) have shown that BioBridge was considered non-irritant and determined to have less inflammatory reaction than the control articles (catgut suture). These implantation studies have also shown that BioBridge integrates well and safely absorbs in subcutaneous tissue in 6 to 9 months.
5) Based on review of approved surgical meshes and earlier testing of BioBridge, it is not anticipated that implanting this small amount of purified collagen into this treatment area will create additional complications to the damaged lymphatic tissue. None of BioBridge's predicate devices have counter indications for use with patients that have lymphedema. A further review of the larger surgical mesh devices that are made of extracellular matrix and widely used in breast reconstruction surgical procedures for similar patients also did not show any contraindications for lymphedema. Preliminary results from the Dominican Republic pilot study provide assurance about this safety, no complications have been reported to date with 55 devices implanted in 8 subjects. Furthermore, in the preclinical

large animal lymphedema study, there were neither overt complications nor any histological evidence of significant inflammatory reaction or other complications observed with the 120 devices implanted.

6) In this proposed treatment, BioBridge is not intended to replace normal body structure or provide full mechanical strength. The device will be implanted in the surgical area during transplantation in subcutaneous soft tissue and not intended to come in direct contact with damaged lymphatic vessels. Even if the device ends up not providing the mechanical function as intended (fails), it is not expected to be catastrophic or to materially impact the subject. If a failure occurs, the surgical outcome would be expected to be the same as the control group receiving the ALNT procedure without the device, which is the current standard of care for lymphedema patients.

5.2 Availability

The investigational device will be provided by Fibralign Corporation. It will be ordered specifically for each subject and will be shipped directly to the PI/Co-PI.

5.3 Agent Ordering

Request for shipments will be coordinated between Stanford PI/Co-PI and Fibralign Corporation.

Fibralign Corporation
32930 Alvarado-Niles Rd, Suite 350
Union City, CA 94587
xxx-xxx-xxxx

5.4 Agent Accountability

The investigational device will be stored in secure location. Only authorized OR staff and study staff will have access to this device. Study investigators will perform accountability.

6. DOSE MODIFICATIONS

N/A (device)

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

BioBridge is contraindicated for use in any patient with known sensitivity to porcine products. Possible adverse reactions may include, but not limited to contamination, infection, inflammation, allergic reaction, adhesions, and tissue encapsulation. If infection or allergic reaction occurs, the entire matrix may have to be revised or removed.

From October 2015 to Oct 2016, a pilot study was conducted in the Dominican Republic. A total of 55 BioBridge threads were implanted into 8 participants with no reported complications

(Hadamitzky, 2016. [43] Personal communication, Drs M Escarraman and C Hadamitzky, 4 April 2017).

Physical exam will be performed at study visits to assess patient safety. Additionally, the subjects will have direct contact information for use 24 hours/day, 7 days/week to report concerning symptoms. Adverse events will be reported as below.

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v5. 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 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 e-Protocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

7.3 Stopping Rule

The study will be terminated if more than 1 of the first 10 subjects experience an infection related to the BioBridge device. If, after the first 10 subjects are treated, a rate > 10% of BioBridge-related infections is observed, accrual will be paused for a safety review. The Stanford Cancer Institute (SCI) Data Safety Monitoring Committee (DSMC) will be informed of any such review.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Laboratory Correlative Studies

Pre-treatment and post-treatment skin punch biopsies will be performed by PI. All research samples (blood and tissue) will be coded with the study participant identifier, and are collected by research team members. Experimental processing will be performed in PI's research lab in CCSR.

8.1.1 The histologic analysis will be performed by means of immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining of pre and post treatment biopsied tissue will be undertaken. Participants in this study will be consented for skin punch biopsies of the affected arm prior to treatment as well as post treatment (optional for control group). Two 6 mm full-thickness skin punch biopsy specimens will be obtained from the medial aspect of the forearm of affected limb, with an Acu-Punch (Fort Lauderdale, FL) disposable device. Biopsy

specimens will be immediately placed in formalin. Following biopsy, the skin edges will be sutured with a single butterfly suture, and the participant will receive a prophylactic antibiotic regimen of cephalexin 250 mg, 4 times daily for 2 days, or an equivalent regimen (if allergic to cephalexin). Skin punch biopsy will be performed at baseline and at completion (12 months post-treatment).

Paraffin-embedded tissue sections will be stained with hematoxylin-eosin (H&E, Richard-Allan Scientific, USA), goat polyclonal anti-LYVE-1 antibody (1:200, Santa Cruz Biotechnology, USA), 5-LO (Cell Signaling 3289), macrophage (Agilent Dako KP1), or neutrophil (anti-Myeloperoxidase antibody, Ab9535 Abcam); cutaneous lymphatic microvascular area will be quantitated through immunohistochemical staining of podoplanin with anti-D2-40 antibody (Dako IS072). Tissue samples will be sliced in 10 µm-thick sections, and evaluated microscopically.

The impact of treatment on cutaneous histopathology will be evaluated through the use of an empirically-derived scoring system (comprised of dermal thickness, intercellular mucin content, deep dermal collagen content, and perivascular infiltrate); this quantitative assessment was developed by a dermatopathologist. The scoring of the specimens for this study will be performed by a blinded observer. Each characteristic will be weighted equally and each specimen will be assigned a cumulative subscale score (0 to 5) will be summed for a total score (0 to 20); higher scores indicate a greater degree of pathology. A quantitatively higher negative change will indicate a more favorable histological therapeutic response.

8.1.2 Luminex Bead Assays. Blood will be drawn in standard fashion, centrifuged for 10 minutes in 4°C room (CCSR 3112), plasma divided into microfuge tubes, and frozen at −80°C for biomarker analysis.

Custom human 62-plex kits (Affymetrix, Santa Clara, CA) will be utilized according to the manufacturer's recommendations with modifications as described below. Plasma samples will be mixed with antibody-linked polystyrene beads on 96-well filter-bottom plates and incubated at room temperature for 2 hours followed by overnight incubation at 4°C. Plates are vacuum-filtered and washed twice with PBS+0.2% Tween-20, followed by incubation with biotinylated detection antibody for 2 hours at room temperature. Samples are filtered and washed twice as above and re-suspended in streptavidin-PE. After incubation for 40 minutes at room temperature, 2 additional vacuum washes are performed, and the samples are re-suspended in Reading Buffer. Each sample will be measured in duplicate. Plates will be read using a Luminex 200 instrument with a lower bound of 100 beads per sample per analyte.

8.1.3 RNA analysis. Tissue from the skin biopsies may be homogenated in a laboratory blender. These samples will be stored frozen at -80° C, and later analyzed for total RNA content; integrity, up- and down-regulation analysis; and possibly other tests, except the RNA samples will not be used for sequencing analyses.

9. STUDY CALENDAR

	Pre-Study	S1	S 2	S3	Surgery: VLNT with BioBridge (BB) Or VLNT only	T1 Mo. 3	T2 Mo. 6	T3 Mo. 9	T4 Mo. 12	T5 D-10 FU	T6 2-yr FU (BB group Only)	T7 3-yr FU (BB group Only)
Investigational Device (BioBridge Matrix)					X	X	X	X	X	X	X	X
Informed consent	X											
Demographics	X											
Medical history	X											
Concurrent meds	X	X	X	X		X	X	X	X	X	X	X
Physical exam	X	X		X		X	X	X	X	X	X	X
Vital signs	X	X	X	X		X	X	X	X	X	X	X
Height	X											
Weight	X	X	X	X		X	X	X	X	X	X	X
Performance status (ECOG)	X	X				X	X	X	X	X	X	X
CBC w/diff, platelets	X											
Serum chemistry	X											
EKG (as indicated)	X											
Adverse event evaluation		X	X	X		X	X	X	X	X	X	X
Measurements (BIS, VM, calipers)		X	X	X		X	X	X	X	X		
LymQoL survey		X	X			X	X	X	X	X		
Skin punch biopsy (optional for control group)			X						X			
Research labs			X						X			
Radiologic evaluation (Lymphoscintigraphy)				X						X *		
Pregnancy test (as indicated)												

* BioBridge Matrix recipients only.

10. MEASUREMENTS

Primary and Secondary Assessments

For each analysis undertaken, primary and secondary, participants will be randomized into one of two groups: VLNT (control arm) or VLNT-BioBridge (intervention arm). Change in intervention group compared to control group will be evaluated.

10.1 Primary Outcome

To determine whether the addition of the BioBridge scaffold to vascularized lymph node transfer will improve the outcome of surgical treatment of secondary arm lymphedema resulting from treatment of breast cancer. Primary endpoint is % change in (excess) limb volume, from baseline to Month 12, in the intervention group relative to control group. Dispersion (variance) will be assessed as the standard deviation.

The single subject enrolled to this study prior to the December 2019 revision will not be included in the primary outcome analysis nor the final analysis, due to the significant changes in the study.

10.1.1 Relevant Subset

Target population, for both groups, are participants with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.1.2 Measurement Definition

Quantitative assessment of limb volume (mL) of the affected limb at study end (Month 12) will be compared to pre-intervention (baseline) value. Baseline value is defined as the arithmetic mean of measurements obtained at the two qualifying screening visits. Mean difference in limb volume in the intervention group relative to control group at study end (Month 12) following surgical intervention will be evaluated.

10.1.3 Measurement Methods

Limb volume quantification, of affected and non-affected limbs, will be performed using circumferential measurements of the limb, at 4 cm intervals beginning at the wrist. The limb volume will be determined using a truncated cone formula{(volume = $\pi h(R^2 + Rr + r^2)/3$, let h be the height, R the radius of the lower base, and r the radius of the upper]. Limb circumference, for calculation of limb volumes, will be serially measured at screening evaluations and in follow-up to study end (Month 12). For quantitative analysis, the volume of the ipsilateral arm will be expressed as a percentage of the unaffected arm volume and of the pre-treatment volume of the ipsilateral arm.

10.1.4 Measurement Time Points

Limb circumference, for calculation of limb volumes, will be measured at screening evaluations to scheduled follow-up visits (Month 3, 6, 9, 12). The primary endpoint will be assessed at time point, 12 months.

10.1.5 Response Review

Results will be evaluated by a designated, independent, blinded reviewer.

10.2 Secondary Outcome

Change in measurement of dermal thickness, as measured by caliper skin fold thickness, from

baseline to Month 12, in the intervention group relative to control group.

10.2.1 Relevant subset

The target population are participants with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.2.2. Measurement Definition

Measurement of dermal thickness at study end (Month 12) will be compared to pre-intervention (baseline) value. Baseline value is defined as the arithmetic mean of values (with standard deviation) obtained at the two qualifying screening visits. Mean difference in dermal thickness measurement in the intervention group relative to control group at study end (Month 12) following surgical intervention will be evaluated. Dispersion (variance) will be assessed as the standard deviation.

10.2.3 Measurement Methods

Skin thickness measurements (in mm) will be performed with a Lange skinfold caliper (Beta Technology, Santa Cruz, CA). For each participant, at each assessment, 3 measurements will be obtained: 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 location is noted (measured from the wrist). These locations will be re-utilized for serial measurements for each follow-up visit. Calipers are calibrated prior to each use. Assessor will be blinded to treatment status. Pre and post-surgical aggregate scores of lymphedema-affected limb will be utilized and reported.

10.2.4 Measurement Time Points

Skin thickness measurements will be performed at screening evaluations to scheduled follow-up visits (Month 3, 6, 9, 12).

10.2.5 Response Review

Results will be evaluated by a designated, independent, blinded reviewer.

10.3 Exploratory Objectives and Endpoints

Exploratory Outcomes have not been defined for ClinicalTrials.gov.

10.3.1 Change in lymphedema Quality of Life (LymQOL)

Change in lymphedema Quality of Life (LymQOL) aggregate score, from screening evaluations to scheduled follow-up visits (Month 3, 6, 9, 12).

10.3.1.1 Relevant Subset

The target population is comprised of patients with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.3.1.2 Measurement Definition

LymQoL survey is a validated, self-reported outcome questionnaire, developed by experienced healthcare professionals in the lymphedema service in the UK (Keeley, *et al*, 2010). Questions cover four areas: symptoms, body image/appearance, function and mood. Answers are scored 1 to 4 (less severe to severe). LymQoL survey will be used to assess change as a result of intervention. Change = Month 12 aggregate score - baseline aggregate score). Baseline score is defined as the arithmetic average of values obtained at the 2 qualifying screening visits.

10.3.1.3 Measurement Methods

Participants will complete the LymQoL survey at each visit, starting with S1 visit through Month 12. The LymQoL will be completed prior to performance of efficacy assessments (volume measurements, skin caliper measurements, L-Dex BIS).

10.3.1.4 Measurement Time Points

LymQoL surveys will be administered at baseline to Month 12 follow-up visit (Month 3, 6, 9, 12).

10.3.1.5 Response Review

Results will be evaluated by a designated, independent, blinded reviewer.

10.3.2 Change in lymphatic flow function

Change in lymphatic flow function by serial radionuclide lymphoscintigraphy (LSG).

10.3.2.1 Relevant Subset

The target population are participants with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.3.2.2. Measurement Definition

For LSG, a significant improvement in the scintigraphic uptake of the transplanted LN (continuous variable); improvement in the uptake of the radionuclide in a defined region-of-interest (ROI) within the proximal LN drainage pathway (axilla); improvement in the reduction of scintigraphic density in a defined ROI for dermal backflow, and improvement in the disappearance rate constant from the injection site.

10.3.2.3 Measurement Methods

LSG: Universal protocol (correct patient, site, procedure) will be adhered to prior to instigation of procedure. After bilateral fingers webs are prepped in a sterile manner, technetium 99m-labeled colloid (radioactive tracer) is injected intradermally around the web spaces of the second and fourth digits (total of 4 point injections). Immediate and delayed (2 hours and 4 hours) static planar images are obtained.

10.3.2.4 Measurement Time Points

LSG will be performed at baseline and 12 months post procedure

10.3.2.5 Response Review

LSG will be evaluated by the co-investigator, Andre Iagaru, MD, Chief of Nuclear Medicine, who will be blinded the subjects' treatment status.

10.3.3 Change in L Dex bioimpedance

Change in L-Dex bioimpedance spectroscopy (L-Dex BIS), from baseline to Month 12, in the intervention group relative to control group.

10.3.3.1 Relevant Subset

The target population are participants with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.3.3.2 Measurement Definition

L-Dex BIS value of the affected limb at study end (Month 12) will be compared to pre-intervention value. Baseline value is defined as the arithmetic average of values obtained at the two qualifying screening visits. Mean difference L-Dex BIS values in the intervention group relative to control group at study end (Month 12) following surgical intervention will be evaluated.

10.3.3.3 Measurement Methods

L-Dex BIS utilizes transcutaneous transmission of a very low frequency, subliminal electrical current to determine extracellular fluid volume. It is a form of widely used body composition analysis.

After cleaning the skin (to remove body oils), electrodes are placed on top of the skin of the limb being measured (for arms, at wrist and top of arm). A 4-electrode configuration will be used to non-invasively assess the extracellular and intracellular fluid contents of the limb. The probes are attached to the electrode itself. Measurement setting is selected and the bioimpedance at an extrapolated frequency = 0 (R_0) correlates to the volume of extracellular fluid. The applied current used is 200uA RMS, at a variable frequency of 4 kHz to 1000 kHz.

Participant data will be entered into the device software (encrypted research-dedicated laptop). Data will be analyzed according to Cole theory, using the manufacturer's software (ImpediMed Ltd.), to provide values for a bioimpedance ratio (Ro), the resistance of the extracellular fluid including lymph, $R\infty$ the resistance of total tissue fluid and Ri, the resistance of the intracellular fluid. For unilateral lymphedema, the ratio of Ro in the affected:unaffected limbs will be analyzed as a measure of the bioimpedance attributable to the extracellular fluid content. Ro level of 1.034 is considered normal; values ≥1.034 are considered abnormal. Efficacy profile in lymphedema has been widely documented and this technology is currently utilized for subclinical lymphedema screening of the relevant cancer survivor population in the Stanford Cancer Institute.

10.3.3.4 Measurement Time Points

L-Dex BIS will be performed at screening evaluations to scheduled follow-up visits (Month 3, 6, 9, 12).

10.3.3.5 Response Review

Results will be evaluated by a designated, independent, blinded reviewer.

10.3.4 Change in cutaneous histological architecture

Change in cutaneous histological architecture, from baseline to Month 12, in the intervention group.

10.3.4.1 Relevant Subset

The target population are participants with unilateral upper extremity lymphedema, secondary to breast cancer treatment, who plan to undergo VLNT.

10.3.4.2 Measurement Definition

Quantitative assessment of paired histological specimens of lymphedema skin pre- and post-intervention. The impact of intervention on cutaneous histopathology will be evaluated through the use of an empirically-derived scoring system (comprised of dermal thickness, intercellular mucin content, deep dermal collagen content, and perivascular infiltrate); this quantitative assessment was developed and will be performed by a dermatopathologist. Each characteristic will be weighted equally and each specimen will be assigned a cumulative subscale score of 0 to 5 (normal to severe). The scores were summed for a total score (range: 0 to 20) which is presented here. Higher scores indicate a higher degree of pathology.
A quantitatively higher negative change indicates a more favorable therapeutic response in the histology.

10.3.4.3 Measurement Methods

Standard protocol (correct patient, site, procedure) will be adhered to prior to instigation of surgical procedures. After consent is obtained and completion of time-out, area will be prepped in a sterile manner, area anesthetized, and skin punch biopsies of the affected arm will be performed (optional for control group) by PI. Two 6 mm full-thickness skin punch biopsy specimens will be obtained from the medial aspect of the forearm of affected limb, with an Acu-Punch (Fort Lauderdale, FL) disposable device. Biopsy specimens will be immediately placed in formalin, or an OCT (optimal cutting temperature compound), or frozen for RNA analysis (will not be used for sequencing analysis). Skin punch biopsy will be performed at baseline and at Week 48 visit.

Paraffin-embedded tissue sections will be stained with hematoxylin-eosin (H&E, Richard-Allan Scientific, USA); goat polyclonal anti-LYVE-1 antibody (1:200, Santa Cruz Biotechnology, USA); cutaneous lymphatic microvascular area will be quantitated through immunohistochemical staining of podoplanin with anti-D2-40 antibody (Dako IS072). Tissue

samples will be sliced in 10 µm-thick sections, and evaluated microscopically.

The impact of treatment on cutaneous histopathology will be evaluated through the use of an empirically-derived scoring system (comprised of dermal thickness, intercellular mucin content, deep dermal collagen content, and perivascular infiltrate); this quantitative assessment was developed by a Stanford dermatopathologist published lymphedema studies. The dermatopathologist for this investigation will be blinded to participant treatment status. Each characteristic will be weighted equally and each specimen will be assigned a cumulative subscale score (0 to 5) will be summed for a total score (0 to 20); higher scores indicate a greater degree of pathology. A quantitatively higher negative change will indicate a more favorable histological therapeutic response.

10.3.4.4 Measurement Time Points

Pre- and post-intervention (Month 12) skin punch biopsies, of affected limb will be performed.

10.3.4.5 Response Review

Histology will be assessed by a dermatopathologist blinded to the participant's treatment status.

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 (eg, 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 his 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 Oncore database system and will be maintained by Principal Investigator and his research team. CRFs (paper documents) will be kept in a secure location, not accessible to the public.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

This study is open-label due to visibly apparent differences between the recipients of the surgery.

Primary Endpoint:

The primary outcome is the post-surgical % change in excess limb volume, measured at 12 months, following the surgical procedure.

Secondary Endpoint:

For secondary endpoint, the mean response of the intervention group will be compared to the control group, for change in measurement of dermal thickness, as measured by caliper skin fold thickness, from screening evaluations to Month 12.

12.1.1 Randomization

A blocked randomization design will be used for randomization. Sixty subjects will be randomized in a 4:1 ratio to the intervention arm (VLNT + BioBridge) or control arm (VLNT alone).

12.2 Interim analyses

Not planned.

12.3 Descriptive Statistics and Exploratory Data Analysis

Not planned.

12.4 Primary Analysis

Limb circumference of the affected and unaffected limbs of each participant will be measured for calculation of the volume of the arm through the truncated cone formula. [23] Circumferential measurements of each limb will be obtained with a low stretch gauged tape measure applied at specified intervals of 4 cm (starting from the 3rd metacarpal head) along the axis of the arm, beginning at the wrist. Anatomic landmarks are recorded to facilitate reproducibility between pre- and post-surgical measurements. This method is inexpensive, reproducible, and easily applied. These measurements will be performed at initial evaluation and at each scheduled follow-up visit. The pre-surgical baseline volume for each limb will be defined as the average of the two measurements obtained on study day S1 and study day S2. For quantitative analysis, the excess volume of the limb is defined as the arithmetical difference between the affected

limb (L_{pre}) and the contralateral normal limb (C_{pre}) and the % excess volume at the beginning of the study is defined by:

$$100\% * [(L_{pre} - C_{pre})/C_{pre}].$$

The primary outcome is defined the post-surgical % change in excess limb volume:

$$100* \{1 - [(L_{post} - C_{post})/(L_{pre} - C_{pre})]\}$$

Analysis Population

Participants are randomized into 1 of 2 arms, the intervention arm (VLNT with BioBridge) or control arm (VLNT-only). The primary analysis will include all participants who have completed all required study visits. However, for participants who do not complete the study, their data will be censored and analyzed with the current population. Treatment summary and adverse event data will also be provided for each treatment category separately.

Efficacy and safety analyses will be performed on all participants who have undergone VLNT (with or without BioBridge). The primary analysis will include all participants who have completed all required study visits and procedures. However participants who do not complete the study will be censored and their data will be analyzed, along with ongoing participants. Data including adverse event data will be captured for each treatment category separately.

Analysis Plan

The final analysis will be undertaken after the final enrolled participant completes all planned study events. At that time, the data will be unblinded and the analyses will be undertaken as described above. There will be multiple analysis populations. The primary analysis will use all subjects enrolled and assigned to treatment, categorized by their planned treatment. 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.

Generalized linear mixed effects models are maximum likelihood based methods that are ideal for handling missing outcome data. More specifically, in prospective clinical trials such as the one proposed, a major concern is loss to follow up. The model we have proposed accommodates anticipated loss to follow up and allows us to analyze subjects treated in the study even if they are lost to follow up, ie, if a subject is lost to follow up, the subject is included in the analysis if at least one post-baseline value is recorded prior to loss to follow up). This model relies on a flexible assumption about the missing data, namely that the missing values are missing at random (ie, that missingness is related to observed values only such as baseline volume values and treatment arm). Given the nature of the data generated, we believe this to be a reasonable assumption.

To allow for the possibility that the response variable may not have a linear relationship with the time variable, we will treat time as a categorical variable to allow time to have a non-linear relationship with the response. The categorical time variable can take on values of 3, 6, 9, or

12 months. We will conduct the primary analysis to evaluate whether there is a difference between the treatment and control arms in the volume reduction of the affected limb at 12 months. We will fit the linear mixed effects model below assuming an AR(1) covariance structure. We will evaluate the sensitivity of our assumption about the covariance structure by assuming an unstructured covariance in a sensitivity analysis. Let Y_{ij} represent the reduction in volume of the affected limb for subject *i* at time *j* (for j = 3, 6, 9, and 12 months), γ_i be the random intercept for subject *i*, ϵ_{ij} be the error for subject *i* at time *j*, trt_i be an indicator for whether subject *i* is in the BioBridge arm, and $m3_{ij}$; $m6_{ij}$; $m9_{ij}$; $m12_{ij}$ be indicators for whether a measurement on subject *i* at time *j* was taken at Month 3; 6; 9; or 12, respectively. To assess whether there is a volume reduction of the affected limb at 12 months, we will test the null hypothesis that $\beta_1 + \beta_7 = 0\beta_1 + \beta_7 = 0$ vs the alternative hypothesis of $\beta_1 + \beta_7 \neq 0$.

$$Y_{ij} = \beta_0 + \gamma_i + \beta_1 trt_i + \beta_2 * m6_{ij} + \beta_3 * m9_{ij} + \beta_4 * m12_{ij} + \beta_5 * m6_{ij} * trt_i + \beta_6 * m9_{ij} * trt_i + \beta_7 * m12_{ij} * trt_i + \epsilon_{ij}$$

We will also perform a sensitivity analysis where we only include subjects with complete data who can provide quantitative changes. Such an analysis relies on an assumption that the data are missing completely at random, an assumption that we do not feel is practical in most clinical trial settings and certainly not here. The motivation for performing this analysis; however, allows us to assess how robust our findings are to deviations in our assumptions of missingness. A final sensitivity analysis will be performed where we impute data using multiple imputation techniques that rely on an assumption that the data are NOT missing at random. More specifically, our imputation model will assume those with missing values in the treatment arm are less likely to have a salutary treatment effect. Such sensitivity analyses will provide a context in which we can appropriately interpret the primary findings. In this sensitivity analysis, we will assume a range of volume reductions in the treatment arm (eg, 90%; 75%; 50% of the reduction observed in the control arm). If we find a statistically significant reduction in the treatment arm as compared to the control arm, the range of reductions will be chosen such that the range covers the “tipping point” where the difference in the two arms is no longer statistically significant. This “tipping point” analysis is in place of a worst-case sensitivity analysis because there is not a clearly defined “worst-case” in a non-binary outcome. Our sensitivity analysis will follow the advice given in “Strategy for intention to treat analysis in randomized trials with missing outcome data.” [25]

12.5 Secondary Analysis

For secondary endpoint, change in measurement of dermal thickness (screening evaluations to Month 12), as measured by caliper skin fold thickness, the mean response of the BioBridge recipients to ALNT only participants will be compared. The statistical methods used for the secondary endpoints will follow the methods used for the primary endpoints.

12.5.1 Analysis Population

N/A; no subset analysis

12.5.2 Analysis Plan

The same analysis plan will be used for secondary endpoint as was used for the primary endpoint.

Exploratory Endpoints and Outcomes

L-Dex: Multiple frequency bioelectrical impedance analysis (MFBIA) will be performed with the impedance device. This tool utilizes multiple frequency electrical current (1 kHz to 500 kHz) to determine extracellular fluid (ECF) volume. At low frequency (< 50 kHz) current does not penetrate the cell membrane into the intracellular space, as opposed to high frequency current (> 200 kHz). As a result, intracellular and extracellular volumes can be measured separately. [26-29] An impedance value, the L-Dex score, can then be calculated which is inversely proportional to the fluid content of the compartment. This noninvasive approach has been evaluated for its capacity to detect subtle disease and to predict the advent of clinically relevant edema. [30-32] MFBIA is predicted to have the requisite sensitivity and specificity to detect the feasibly small differences in retained interstitial fluid that might discriminate the therapeutic responses of the treated patients. MFBIA will be measured at screening evaluations and at each follow-up visit. A standardized quantitative score, the L-Dex score, will be generated for the ipsilateral and contralateral limb. [30] This will be done as specified in the treatment plan above. It has been established that a 10-point change in L-Dex score corresponds to 3 standard deviation(SD) range from the mean. [30,31] See Figure 3 below.

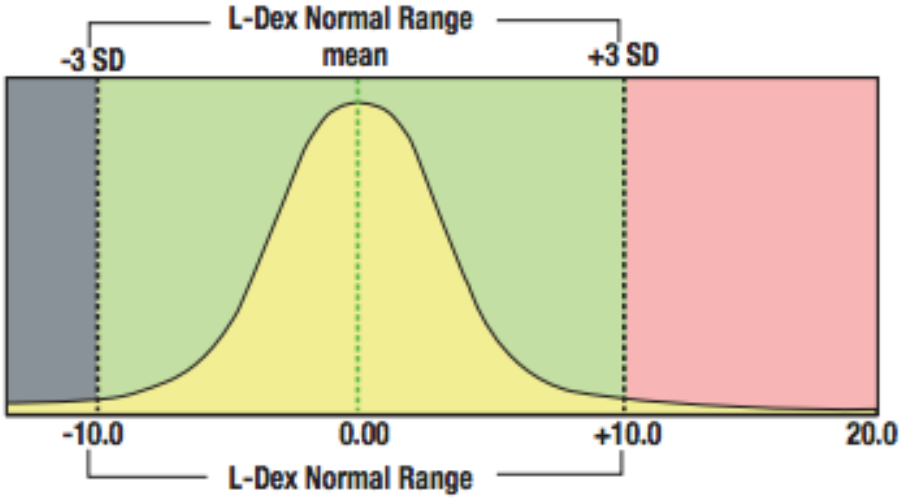


Figure 3. 10-point change in L-Dex score corresponds to 3 SD range from the mean **(**obtained from ImpediMed L-DEX technical bulletin**).** [30,31]

Change in lymphatic flow function by serial radionuclide lymphoscintigraphy (LSG):

Significant improvement in the scintigraphic uptake of the radionuclide in a defined ROI within the proximal LN drainage pathway (axilla); improvement in the reduction of scintigraphic density in a defined ROI for dermal backflow, and improvement in the disappearance rate constant from the injection site.

Change in the lymphedema Quality of Life (LymQOL) score:

Greater improvement in the aggregate score on the validated instrument.

Change in quantifiable skin histopathology, optional for control group: greater reduction in dermal-epidermal thickness, greater reduction in dermal collagen thickness, and greater improvement in lymphatic vascular size and density.

12.6 Sample Size

12.6.1 Accrual estimates

Dr Rockson directs a national referral center for lymphatic disorders and approximately 600 new patients with clinically significant lymphedema are seen in on a yearly basis. Some of these patients are referred from the breast clinic but majority comes from local and regional physician offices. Dr Nguyen performs 15 to 19 vascularized lymph node transfers yearly (of subjects who meet the eligibility criteria). We expect to be able to accrue subjects to this study within 48 months. Once study has been approved and is enrolling, in order to facilitate enrollment in the allotted time frame, we plan to add 3 subsites in order to meet accrual goal.

12.6.2 Sample size justification

In order to successfully test the null hypothesis, we have performed sample size calculations. After careful review of the published literature, we are able to identify a study in which the effect of the surgery on limb circumference (as a surrogate for limb volume) has been quantitated and for which a published effect size of 51% reduction in limb volume with standard deviation of 19% is available for standard care. [33] We have utilized this published value (SD = 19%) to calculate our power in relationship to the detectable alternative If we allow for a 4:1 ratio of VLNT in intervention: control arms, and successful project completion of the protocol by 48 VLNT with BioBridge subjects and 12 controls (VLNT without BioBridge), we have utilized an alpha = 0.05 and a power = 0.9 to predict that the detectable alternative would be ± 20%.

12.6.3 Effect size justification

An additional volume reduction of 20% ascribable to the BioBridge (versus VLNT only) would represent a substantial and clinically relevant amelioration in the surgical outcome when compared to current, standard VLNT without BioBridge supplementation. This magnitude of treatment differential has been demonstrated for the BioBridge in lymphedema, both in our published large animal study as well as in pilot observations of the surgical effect of the BioBridge performed in the Dominican Republic (unpublished observations).

12.7 Criteria for future studies

Future studies are not planned at this time.

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APPENDICES

APPENDIX A: Participant Eligibility Checklist

Protocol Information

Protocol Name: Prospective Evaluation of a Surgical Solution for Breast Cancer-Associated Lymphedema

E-Protocol number: IRB-37161 / BRS0095

Principal Investigator: Stanley Rockson, MD

Participant Information

Participant Name/ID:

Date of Birth:

Gender: ☐ Male ☐ Female

Study Information

SRC-approved ☐ IRB-approved ☐ Contract signed ☐

Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB-approved protocol)	**Yes**	**No**	**Supporting Documentation**
1. Ages 18 to 75 years (inclusive)			
2. Eastern Cooperative Oncology Group (ECOG) Performance Status 0 to 2			
3. Life expectancy > 2 years			
4. Acquired (secondary) upper limb lymphedema (secondary to breast cancer treatment)			
5. The participant must be eligible for surgical intervention.			
6. Swelling of 1 limb that is not completely reversed by elevation or compression			
7. Stage II or greater lymphedema at screening, based on the International Society of Lymphology (ISL) staging system			
8. Participants must have no evidence of disease (NED), have completed breast cancer therapy 3 years prior to enrollment; use of endocrine therapy is allowed.			
9. Completion of a full course of complete decongestive therapy (CDT), according to ISL guidelines at least 8 weeks prior to screening, including use of compression garments for at least 8 weeks without change in regimen			

10. Willingness to maintain a stable regimen of self-care, including use of compression garments from screening through the entire study duration (through the safety follow-up visit). Self-bandaging, use of nighttime compression garments, and intermittent pneumatic compression devices are allowed, but the procedures and regimens are expected to remain consistent from screening though the entire study duration.			
11. Consistent use of an appropriately-sized compression garment for daytime use.			
12. Two consecutive measurements of limb volume (LV) in the affected arm, taken at least 1 day apart during the screening period, must be within 10% of each other. A maximum of 3 measurements can be taken. Affected limb volume ratio must be > 20% (compared to unaffected limb); volume measurements will be performed and volume ratio will be calculated at S1 and S2 visit.			
13. Evidence of abnormal bioimpedance ratio, if feasible, based upon unilateral disease: L-Dex > 10 units; bioimpedance performed at S1 and S2			
14. Willing and able to comply with all study procedures, including measurement of skin thickness using skin calipers			
15. Willing and able to understand, and to sign a provide written informed consent			
Exclusion Criteria (From IRB-approved protocol)	**Yes**	**No**	**Supporting Documentation**
1. Edema arising from increased capillary filtration will be excluded (venous incompetence).			
2. Inability to safely undergo general anesthesia and/or perioperative care related to vascularized lymph node transfer			
3. Concurrent participation in a clinical trial of any other investigational drug or therapy, regardless of indication, within 1 month before screening or 5 times the drug's half-life, whichever is longer.			
4. Recent initiation of (≤ 8 weeks), or intention to initiate, CDPT or maintenance physiotherapy for lymphedema at any time during the duration of the study			
5. Other medical condition that could lead to acute limb edema, such as (but not limited) to acute venous thrombosis			

6. Other medical condition that could result in symptoms overlapping those of lymphedema in the affected limb (eg, pain, swelling, decreased range of motion)			
7. History of clotting disorder (hypercoagulable state)			
8. Chronic (persistent) infection in the affected limb			
9. Any other infection (unrelated to lymphedema) within 1 month prior to screening			
10. Currently receiving chemotherapy or radiation therapy			
11. Current evidence, or a history of malignancy within the past 3 years (except for non-melanoma skin cancer or cervical cancer in situ treated with curative intent). If the participant has undergone cancer treatment, this must have been completed > 3 years prior to enrollment.			
12. Current evidence of any high risk for recurrence of breast cancer [eg, Stage III or IV; estrogen receptor (ER) / progesterone receptor (PR) / HER-2 negative (ie, "triple-negative") cancer; locally-advanced disease; inflammatory breast cancer; > 3 positive axillary lymph nodes; extracapsular nodal extension; invasive micropapillary breast carcinoma; or if performed, genetic testing, eg, BRCA1; BRCA2; Oncotype DX (high-risk recurrence score); or Mammaprint (poor risk signature) indicating a high risk for breast cancer recurrence			
13. 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			
14. 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			
15. Absolute neutrophil count < 1500 mm^3 at screening			
16. Hemoglobin concentration < 9 g/dL at screening			
17. Known sensitivity to porcine products			
18. Hypersensitivity to iodine			
19. Pregnancy or nursing			
20. Substance abuse (such as alcohol or drug abuse) within 6 months prior to screening			
21. Any reason (in addition to those listed above) that, in the opinion of the investigator, precludes full participation in the study			

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

_____ _____

Second Reviewer Signature Date

_____

Printed Name

_____ _____

Study Coordinator Signature Date

_____

Printed Name

APPENDIX B: Lymphedema Quality of Life Survey

Lymphoedema Quality of Life Study (LYMQOL) LEG

If any of the items are not applicable to you, please write N/A in the relevant answer box(es).

(1) Has your swollen leg(s) affected:				
	Not at all	A little	Quite a bit	A lot
a) your walking				
b) your ability to go up and down stairs				
c) your ability to bend, e.g. to tie shoelaces or cut toenails				
d) your ability to kneel				
e) your ability to stand				
f) your ability to get into/out of a car				
g) Your ability to get on/of public transport, e.g. trains/buses				
h) your ability to get up from a chair				
i) your ability to drive a car				
j) your occupation				
k) your ability to do housework				
(2) Does the swelling affect your leisure activities/social life?				

Please give example(s) of this.

.....

.....

(3) How much do you have to depend on other people?				
(4) How much do you feel the swelling affects your appearance?				
(5) How much difficulty do you have finding clothes to fit?				
(6) How much difficulty do you have finding clothes you would like to wear?				
(7) Do you have difficulty finding shoes to fit?				
(8) Do you have difficulty finding socks/tights/stockings to fit?				
(9) Does the swelling affect how you feel about yourself?				
(10) Does it affect your relationship with your partner?				
(11) Does it affect your relationships with other people?				
(12) Does your lymphoedema cause you pain?				
If so, do you have pain in the foot/feet				
leg/legs				
hip(s)				
back				
elsewhere — if so, where?				
(13) Do you have any numbness in your swollen leg(s)?				
(14) Do you have any feelings of 'pins and needles' or tingling in your swollen leg(s)				
(15) Does (do) your swollen leg(s) feel weak?				
(16) Does (do) your swollen leg(s) feel heavy?				
(17) Does (do) your swollen foot (feet) feel 'old'?				
(18) Have you had any leakage of fluid from your leg(s)				
In the past week				
(19) Have you had trouble sleeping?				
(20) Have you had difficulty concentrating on things, e.g. reading?				
(21) Have you felt tense?				
(22) Have you felt worried?				
(23) Have you felt irritable?				
(24) Have you felt depressed?				

(25) Overall, how would you rate your quality of life at present? Please mark your score on the following scale:

Poor	0	1	2	3	4	5	6	7	8	9	10	Excellent

Lymphedema Quality of Life Scoring System

LYMQOL ARM-Scoring System
Lymphoedema Quality of Life Tool

The score for the individual responses are given below. If the item is not scored and left blank or not applicable this is scored with a 0. Domain totals are calculated by adding the individual scores and dividing the total by the number of questions answered. (If >50% of questions per domain are not answered this cannot be calculated and =0).
The four domains and their corresponding questions are: Function 1 (a–h), 2, 3;
Appearance 4, 5, 6, 7, 8; Symptoms 9, 10, 11, 12, 13, 14; and Mood 15, 16, 17, 18, 19, 20.
Overall quality of life (Q21) is scored as the value marked by the patient, between 0–10.

(1) How much does your swollen arm affect the following daily activities?	Not at all	A little	Quite a bit	A lot
a) occupation	1	2	3	4
b) housework	1	2	3	4
c) combing hair	1	2	3	4
d) dressing	1	2	3	4
e) writing	1	2	3	4
f) eating	1	2	3	4
g) washing	1	2	3	4
h) cleaning teeth	1	2	3	4

(2) How much does it affect your leisure activities/social life?	1	2	3	4

Please give example(s) of this.

.....

.....

(3) How much do you have to depend on other people?	1	2	3	4
(4) How much do you feel the swelling affects your appearance?	1	2	3	4
(5) How much difficulty do you have finding clothes to fit?	1	2	3	4
(6) How much difficulty do you have finding clothes you would like to wear?	1	2	3	4
(7) Does the swelling affect how you feel about yourself?	1	2	3	4
(8) Does it affect your relationships with other people?	1	2	3	4
(9) Does your lymphoedema cause you pain?	1	2	3	4
(10) Do you have any numbness in your swollen arm?	1	2	3	4
(11) Do you have any feelings of 'pins and needles' or tingling in your swollen arm?	1	2	3	4
(12) Does your swollen arm feel weak?	1	2	3	4
(13) Does your swollen arm feel heavy?	1	2	3	4
(14) Do you feel tired?	1	2	3	4
In the past week	1	2	3	4
(15) Have you had trouble sleeping?	1	2	3	4
(16) Have you had difficulty concentrating on things, e.g. reading?	1	2	3	4
(17) Have you felt tense?	1	2	3	4
(18) Have you felt worried?	1	2	3	4
(19) Have you felt irritable?	1	2	3	4
(20) Have you felt depressed?	1	2	3	4
(21) (24) Overall, how would you rate your quality of life at present? Please mark your score on the following scale:	1	2	3	4

Poor	0	1	2	3	4	5	6	7	8	9	10	Excellent