Lumicell Protocol Number: CL0007
Lumicell Study Identification: CL0007_Breast
Protocol Version: 08
Protocol Version Date: 07APR2021

Study Title: PIVOTAL STUDY OF THE LUM IMAGING SYSTEM FOR ASSISTING INTRAOPERATIVE DETECTION OF RESIDUAL CANCER IN THE TUMOR BED OF FEMALE PATIENTS WITH BREAST CANCER

Funded in part by NIH Grant 5R44CA211013-02

System: LUM Imaging System (LUM015 imaging agent and LUM Imaging Device)
IDE # G140195/S039

Study Sponsor and Investigational Product
Supplier: Lumicell, Inc.
Contact information:
Jorge Ferrer, Ph.D.
Senior Vice-President, Clinical Research and Strategy
jmferrer@lumicell.com
Phone: 617-571-0592

Protocol Lead Principal Investigator:
Barbara L. Smith, MD, PhD
Director, Breast Program
Massachusetts General Hospital
55 Fruit Street
YAW9
Boston, MA 02114
Phone: 617-726-6500
Fax: 617-724-1079

Medical Monitor: Karl Gallegos, M.D.
kgallegos@lumicell.com

TABLE OF CONTENTS

1 PURPOSE

The objective of this prospective, multi-center, two-arm randomized, blinded study is to demonstrate the safety and efficacy of the LUM Imaging System (LUM015 imaging agent in conjunction with the LUM Imaging Device and decision software), in identifying residual cancer in the lumpectomy bed of female breast cancer patients. The size of this study is driven by the necessary number of truth standard positive events; therefore, we propose an event-driven clinical study. Patients will be enrolled until 70 truth standard positive events are reported, up to a maximum of 450 patients.

2 SUMMARY OF ENDPOINTS

2.1 Three co-primary endpoints

- Ratio of patients who have **residual cancer** found in at least one Lumicell-guided shave (also known as therapeutic shave) among all patients. **Residual cancer** is defined as tumor found by pathology in a therapeutic shave after the SOC surgical procedure is completed; that is, tumor that current SOC surgery failed to remove.
- Instrument diagnostic accuracy measured as two separate endpoints of **sensitivity** and **specificity** on a per-tissue basis.

2.2 Secondary endpoints

a. Proportion of patients with positive margins after standard of care breast-conserving surgery who have a LUM Imaging System signal in the cavity above the threshold as defined by the tumor detection algorithm (i.e., a positive LUM signal).
b. Proportion of subjects with pathology-positive margins after standard of care breast-conserving surgery for whom additional LUM Imaging System-guided shaves resulted in pathology-negative margins among patients with positive margins. Note: This endpoint also estimates the potential reduction in re-excision surgeries following standard of care breast-conserving surgery that resulted from a positive SOC margin.
c. Proportion of subjects with pathology-positive margins after standard of care breast-conserving surgery for whom additional LUM Imaging System-guided shaves resulted in pathology-negative margins among all patients.
d. Ratio of patients with negative margins after the SOC procedure who have residual cancer found in at least one Lumicell-guided (or therapeutic) shave among patients with negative margins.
e. Ratio of patients with negative margins after the SOC procedure who have residual cancer found in at least one Lumicell-guided (or therapeutic) shave among all patients
f. Mean and median incremental volume (in cubic centimeters) of tissue removed from therapeutic shaves: For each patient, absolute shave volume will be calculated as length (c) × width (c) × depth (c) for each therapeutic shave with the sum by patient of all therapeutic shaves. Subjects with no therapeutic shaves taken will have a therapeutic shave volume of 0cc.
g. Mean and median contribution of therapeutic shave volume to total tissue removed.
h. Comparison of adverse events in subjects that have at least one therapeutic shave versus subjects that have no therapeutic shaves.

i. Average number of therapeutic shaves taken per subject overall and by type of SOC lumpectomy procedure (i.e., lumpectomy with comprehensive shaves vs. lumpectomy with or without selective shaves).
j. Percent of device failures that led to failure of using the LUM Imaging System to capture data.
k. Rate of re-excision procedures as a result of a positive margin status following SOC (control arm) and LUM Imaging procedures (device arm).
l. Number of re-excisions for each patient
m. Number of re-excisions recommended
n. Collect exploratory data on tissue types found in therapeutic shaves.
o. Collect exploratory data on patient reported outcome measures (PROMs).
p. Adverse events stratified by severity and relatedness to drug/device.
q. Serious adverse events stratified by severity and relatedness to drug/device.
r. Adverse events by preferred term (sorted by descending occurrences for each preferred term).
s. Summary of adverse events, overall and split by expectedness to drug/device.

3 BACKGROUND

3.1 Study disease

For breast cancer lumpectomies, the presence of residual cancer cells left in the tumor bed after initial resection is inferred by post-operative margin assessment of the resected tissue by a pathologist. For invasive carcinoma and ductal carcinoma in situ (DCIS), a positive margin is defined as having tumor present at the inked side of the outermost surface of the lumpectomy specimen [1, 2]. However, according to the Society of Surgical Oncology (SSO), for negative margins of less than 2mm in patients with DCIS, clinical judgment is recommended to determine the need for a second surgical procedure [2]. Positive lumpectomy margins are the most important risk factor for local recurrence of breast cancer [1-5] and dictate that a second surgical procedure be performed to obtain tumor-free margins. The rates of close or positive margins have been reported between 17 to 59% [6-10]. Among the more than 318,000 women expected to be diagnosed with breast cancer in the US in 2017, approximately 200,000 underwent lumpectomy and approximately a third required additional surgery for positive margins [11]. Thus, a diagnosis of positive margins indicates that additional treatments are needed. This places a heavy burden on patients and adds significant cost to the healthcare system.

3.2 Rationale

Achieving negative margins during tumor excision is critical to assure that all or most of the cancer has been removed from the patient. Current standard of care dictates that tissue removed during surgery is analyzed post-operatively for tumor histology and margin assessment by a pathologist as described above. This procedure is time consuming, often requiring 7-10 days of pathology testing after the operation before margin status is known. In addition, this process is prone to sampling errors, as only a finite number of tissue slices can be examined, meaning that only a small fraction of the lumpectomy margin surface is assessed, < 1% of lumpectomy surface area by some estimates [2]. Thus, a safe method to directly assess the entire tumor bed intraoperatively and identify residual microscopic disease for immediate resection intraoperatively would be highly beneficial for patients.

Lumicell has developed the LUM Imaging System, which consists of a fluorescence-based imaging agent (LUM015), a hand-held, wide-field detector (LUM Imaging Device) that can image the lumpectomy cavity in seconds, and a tumor detection algorithm that highlights regions suspected to contain cancer and displays them on a computer monitor. The LUM Imaging system is intended to be used to scan the lumpectomy cavity walls intraoperatively after the resection of the main lumpectomy specimen.

The purpose of the Lumicell Imaging System is to enable surgeons to achieve negative margins during the initial surgery and eliminate or reduce the need for re-excision surgeries when compared with current standard of care procedures. Lumicell's system images the entire lumpectomy cavity surface in vivo, overcoming tissue handling and sampling limitations of standard pathology analysis of resected tissue. The LUM Imaging System provides real-time feedback to the surgeon about the status of the lumpectomy cavity walls.

The LUM Imaging System has been tested in a two-phase Feasibility Study at the Massachusetts General Hospital. There were 15 patients in Phase A and 45 patients in Phase B in the study. Of the 11 Lumicell imaged subjects for whom a pathology positive margin followed SOC, the LUM Imaging Device detected 9 (82%) of the subjects with residual cancer in the cavity. A multi-site Feasibility Phase C study is being conducted to refine and verify the tumor detection algorithm and to complete hands on training of surgeons and clinical staff.

3.3 Definitions

3.3.1 Definitions of excised tissue

- **Main specimen**: also known as the "lump" or main mass, refers to the primary piece of tissue removed during a lumpectomy. Typically, this is the largest piece of tissue removed and it is intended to contain most of the tumor.
- **Shave types**: pieces of tissue removed from the lumpectomy cavity walls after the main specimen is removed. In this study, we define three different types of shaved cavity margins (SCMs) below.
    - **Selective shaves**: shaved cavity margins removed from a specific location of the lumpectomy cavity wall based on intraoperative analysis of the **intact main specimen** by methods including X-ray imaging, ultrasound imaging, palpation, frozen sections or visual examination. Selective shaves precede all other SCMs described below.
    - **Comprehensive shaves**: shaved cavity margins removed from *all* available surfaces of the lumpectomy cavity walls after resection of the specimen based on the SOC for that surgeon/site
    - **Therapeutic shaves**: shaved cavity margins removed following the guidance of the LUM Imaging System; may also be referred to as Lumicell-guided shaves.

3.3.2 Definition of standard lumpectomy procedure used in this protocol

Breast conserving surgeries may vary between surgeons and institutions. However, most surgical practices perform what is defined in this protocol as a "**standard lumpectomy procedure**". This procedure consists of the surgeon's best attempt to remove the main specimen with grossly negative margins and may be followed by the removal of **selective shaves** (see definition above). Some institutions have implemented additional margin management techniques such as comprehensive shaves, as defined above, to try to lower the rates of positive margins and re-

excision [12, 13]. **Table 1** outlines the surgical procedure options that will be allowed in this study to support the primary and secondary endpoints.

Table 1: Surgical procedures to be included in the SOC arm of this clinical study.

SOC procedure	Description	Basis for margin assessment
A. Standard lumpectomy procedure: main specimen followed by selective shaves	Most sites in the US practice this procedure. The main specimen is removed and some selective shaves may be removed based on palpation, X-ray imaging, frozen sections or visual inspection of the **intact main specimen**.	Outermost surface from a given orientation that can come from the main specimen or a selective shave when a shave exists.
B. Standard lumpectomy procedure followed by comprehensive cavity shaves	After the standard lumpectomy procedure, a cavity shave is removed from all the available orientations. A shave from anterior or posterior may not be removed if no breast tissue remains at these surfaces.	Outermost surface from a given orientation that can come from the main specimen or a comprehensive shave when a shave exists.

3.3.3 Definition of endpoints

3.3.3.1 Definition of positive margins

For this protocol, positive margins are defined using the latest consensus from the Society of Surgical Oncology as follows:

- For invasive cancer with or without associated ductal carcinoma in situ (DCIS): cancer cells or DCIS present on ink [1]
- For pure DCIS lesions: DCIS present less than 2 mm from the ink [2]

3.3.3.2 Definition of re-excisions

A re-excision is defined as a surgical procedure performed in the ipsilateral breast following the primary lumpectomy because of positive margins found from the initial lumpectomy procedure. The re-excision procedure can be a separate surgical procedure to excise further breast tissue or a mastectomy.

3.3.3.3 Definition of positive cavity

After the standard lumpectomy procedure, a positive cavity occurs when tumor is found in a therapeutic shave or during a re-excision.

4 PRODUCT DESCRIPTION

4.1 **Intended use**

The LUM Imaging System is a combination product consisting of the LUM015 imaging agent and the LUM Imaging Device. LUM015 is administered to the patient via intravenous injection 4 ± 2 hours prior to the first image recorded. The LUM Imaging Device is intended for use in vivo

following the excision of the main lumpectomy specimen to detect remaining cancer cells after breast conserving surgery.

4.2 Proposed indications for use

The LUM Imaging System is indicated for use in patients undergoing a breast conserving surgery (lumpectomy) to remove breast cancer. The LUM Imaging System is not indicated for those subjects receiving administration of blue (e.g. methylene blue, isosulfan blue) or green (e.g. indocyanine green) dyes for sentinel lymph node mapping procedures prior to the lumpectomy procedure. The LUM Imaging System is intended to be used whenever breast tissue is removed, histopathology evaluation of the tissue is the standard of care and/or it is essential that the tissue margins be examined for completeness of removal using standard surgical procedures. The LUM Imaging System is to be used *in vivo* following the excision of the main lumpectomy specimen to assist in locating residual abnormal tissue, therefore, facilitating tumor resection and potentially creating negative margins on the outermost surfaces of excised tissue.

4.3 LUM015 imaging agent

Lumicell engineered LUM015, a fluorescence-based imaging agent that accumulates within and around cells. In regions enriched with cathepsin enzymes, such as cancer cells, LUM015 is altered to fluoresce when illuminated with 649 nm light. In its nominal state, the fluorescence of LUM015 is suppressed by an internal quencher molecule (QSY21). The presence of cathepsin enzymes is not fully confined to cancer cells; hence, data from this study will help determine the cancer localization properties of LUM015. Once cathepsin enzymes cleave LUM015 at its amino acid backbone, the quencher is released and the fluorescent dye (Cy5) in LUM015 emits detectable fluorescence when it absorbs red light (**Figure 1**). The absorption and emission wavelength maxima for Cy5 are 649 nm and 670 nm respectively. LUM015 employs a fluorescent molecule with excitation and emission in the far red spectrum (> 600 nm wavelength) because in that range light can travel effectively through 1mm of tissue and tissue autofluorescence is minimal, allowing higher sensitivity due to lower background [14].

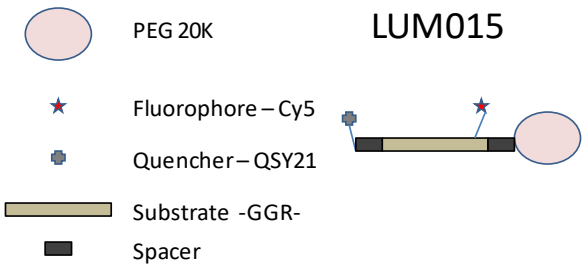


Figure 1: Schematic representation of LUM015.

Cathepsins are a family of enzymes that are involved in the degradation of the extracellular matrix to allow tumor growth and progression. These enzymes are upregulated in most human cancers and are also present in tumor-associated macrophages at the tumor's invasive front [15-19]. Platt et al. showed 60-fold higher cathepsin activity in breast cancer tissue than in healthy breast tissue [20]. Others report over-expression of cathepsin enzymes (B, K, L and D) in ductal carcinoma in situ as well as in invasive carcinomas (lobular and ductal) [16, 20-23]. Also, cathepsin B is typically over-expressed in inflammatory breast cancer, one of the most lethal forms of primary breast cancer. [24, 25]. The peptide sequence used in LUM015 is a pan-cathepsin substrate

meaning that it will be cleaved by multiple enzymes of the cathepsin family. Because high activity of cathepsin enzymes is found in tumor cells and tumor associated macrophages surrounding the tumor at the invasive front, these enzymes provide an excellent marker for activating LUM015 at the tumor margin. The fluorescence of LUM015 is then induced and detected with the LUM Imaging System to identify cancer and cancer-related cells for immediate resection and to distinguish them from adjacent normal tissue.

4.4 LUM Imaging Device

The LUM Imaging Device consists of a computer control unit, monitor and light source mounted on a cart, and a hand-held optical head (LUM002/LUM003). The computer control unit collects, analyzes (based upon Lumicell's detection algorithm) and displays the resulting images gathered by the imaging head in real-time. The light source provides the illumination to excite the fluorescent dye present in LUM015. Light is transferred from the light source to the imaging head using an optical fiber bundle. The imaging head (Figure 2) was designed as a lightweight hand-held tool with a small profile to allow easy maneuverability and limited intrusiveness in the operating room. Because incisions and lumpectomy cavities can vary in size, Lumicell offers two options for the distal end diameter: LUM002, which has a 2.6 cm field of view along with a 3.1 cm outer diameter and LUM003, which has a 1.3 cm field of view and a 1.9 cm outer diameter. The surgeons will be able to choose which device size to use for any given surgery. These two device options were proven to be optically equivalent when imaging freshly excised breast tissue. Both imaging heads have a 45° bend at the distal end for examination of the walls of the lumpectomy cavity.

The hand-held optical head will be used within the sterile surgical field. Consequently, a sterile barrier assembly will be provided to cover the optical head, which comes into contact with both the surgeon and the patient's exposed tumor bed. The sterile barrier assembly is installed on the LUM Optical Head in the OR using sterile procedures.

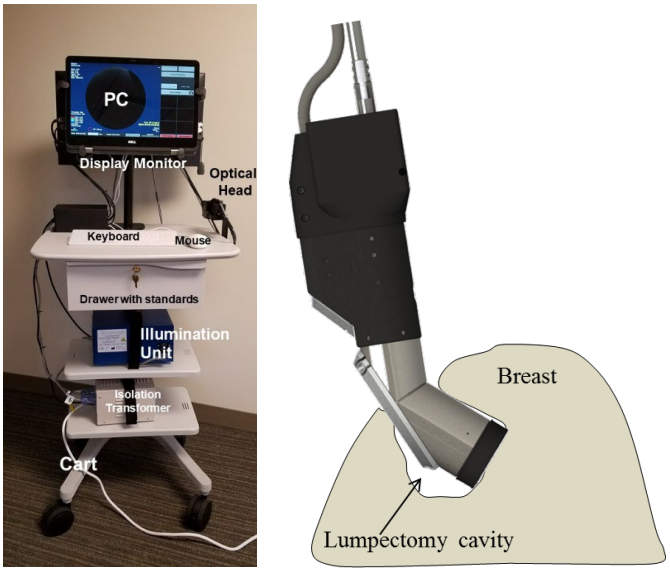


Figure 2: (Left) Photo of the LUM Imaging System. (Right) Rendering of the LUM002 Optical Head scanning a lumpectomy cavity. The sterile cover over the device is not shown.

4.5 LUM decision software

The LUM Imaging System is powered by Lumicell's proprietary decision software. The decision software uses an initial set of images acquired from the lumpectomy cavity after the main specimen is removed to set the tumor detection threshold. Then, while the surgeon scans the lumpectomy cavity walls with the LUM Optical Head, the decision software compares the intensity of an image against the tumor detection threshold and identifies whether a region of the image is suspected to contain tumor. Regions with fluorescence signal above the threshold are highlighted in the computer monitor for the surgeon to see in real-time; that is, there is no delay between the scanning of a region and the display of the results from that region. **Figure 3** shows the user interface and what the surgeon sees during scanning of the cavity walls. The decision software was developed and optimized during Phase A and Phase B of the Feasibility Study in breast cancer patients.

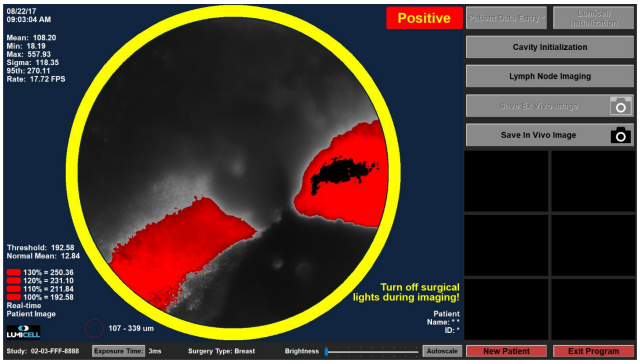


Figure 3: Screen shot of the user interface for the LUM decision software. Regions highlighted in red indicate areas suspect to have tumor.

4.6 Prior clinical studies with the LUM Imaging System in females with breast cancer

Following the designation of a device primary mode of action for the LUM Imaging System combination product by FDA, Lumicell and collaborators at MGH conducted a Feasibility Study in breast cancer patients under an Investigational Device Exemption (IDE). The Feasibility Study was divided into Phase A, Phase B and Phase C as described below, and all phases have been completed. A multi-site Pivotal Study is underway.

4.6.1 Summary of three phases of the Feasibility study (all completed)

- **Phase A (n=15 patients)**: patients were administered with either no LUM015 (5 patients) or a dose of 0.5 mg/kg (5 patients) or 1.0 mg/kg (5 patients) 4 ± 2 hours prior to surgery and the LUM Imaging System was used to image the lumpectomy cavity before and after standard of care cavity shaves. Resected cavity shaves were also imaged. The imaging results were compared with pathology analysis of the cavity shaves. The endpoints were: (1) select a dose of LUM015 for Phase B; (2) determine the parameters of the tumor detection threshold; and (3) acquire safety data. No clinical action was taken by the surgeon based on the imaging results. A dose of 1.0 mg/kg was selected for Phase B and all subsequent clinical studies in breast cancer.
- **Phase B (n=45 patients)**: the tumor detection threshold was implemented and the LUM Imaging System was used to scan the lumpectomy cavity to guide the removal of additional cavity shaves beyond the standard of care cavity shaves. The endpoints were: (1) evaluate and, if necessary, adjust the detection algorithm parameters, (2) adjust, if necessary, the protocol for the pivotal trial, and (3) collect safety data.

- **Phase C (multi-center evaluation, n=234 patients)**: the tumor detection threshold and the LUM Imaging System was evaluated at multiple sites to (1) collect data to refine and verify the tumor detection algorithm, (2) complete hands-on training of surgeons and clinical staff that will be participating in the pivotal study, (3) identify and address any site-specific or user-specific issues for using the LUM Imaging System in breast cancer surgeries, and (4) collect safety and efficacy data.

Once a surgeon was trained in the use of the LUM Imaging System during Phase C of the Feasibility Study, the tumor detection algorithm was locked and this protocol has been initiated, the surgeon may begin enrolling patients for the pivotal study. Any surgeon or site that did not participate in the Phase C study will be required to undergo training and complete a modified proficiency program before starting enrollment for this pivotal study.

No serious adverse events were observed in both Phase A and Phase B of the Feasibility Study. The only adverse event noted in these two phases was a temporary blue discoloration at the injection site for LUM015 that resulted from extravasation of LUM015 during injection (see details in section 11.2) One patient enrolled in the Phase C study, with known hypersensitivity to contrast, experienced an anaphylactic reaction to the LUM015 injection. The patient recovered in <24 hours. No other related serious adverse events have been reported to date.

5 PIVOTAL STUDY DESIGN

5.1 Design Summary

This is a prospective, multi-center, two-arm, randomized, blinded clinical trial. All eligible subjects are injected with LUM015. All eligible subjects shall follow the day of surgery workflow illustrated in Figure 4.

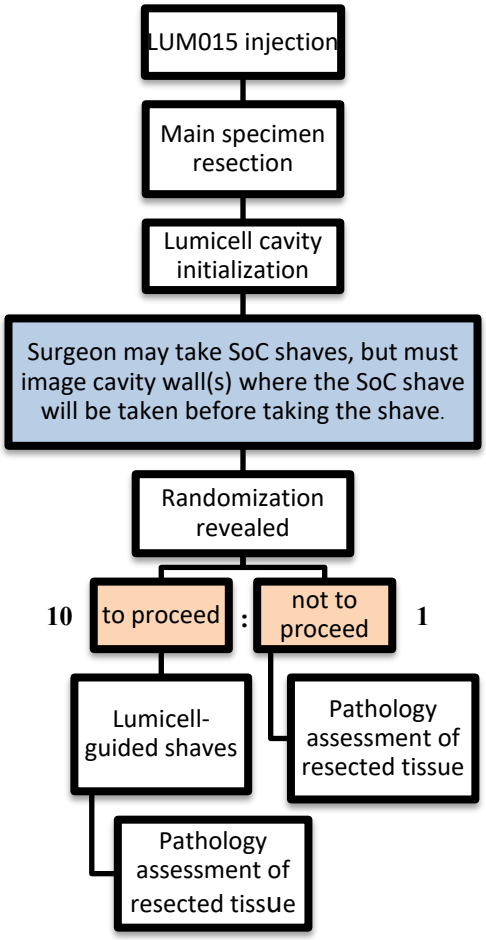


Figure 4: Surgery workflow diagram. A detailed description of each step in the surgical workflow is provided in sections 5.2.3 - 5.2.9 below.

5.2 **Methods**

5.2.1 Establishing a patient in the electronic data capture system (EDC)

All consented patients should be entered into the electronic data capture system prior to surgery.

5.2.2 Collection of baseline Patient Reported Outcome Measures Data

After the patient has consented to participate and has consented to the collection of Patient Reported Outcome Measures (PROMs) Data, prior to surgery start, each enrolled subject should complete this baseline survey collecting data to support an evaluation of PROMs. Baseline evaluations will be electronically collected using the EDC system at any time after the patient consented to participate in the study and prior to their lumpectomy procedure. If the patient has not yet completed their survey at the time of injection, the study site should facilitate and remind the patient to complete their survey. The patient should not complete the baseline survey after the lumpectomy procedure. The completion of the survey is not a requirement to be enrolled into the study. Alternatives to electronic distribution of the surveys may be implemented.

5.2.3 LUM015 Injection and Randomization

All consented and eligible patients will be injected with LUM015 at a dose of 1.0 mg/kg 4 ± 2 hours prior to the first image recorded with the LUM Imaging System during the lumpectomy procedure. After injection of Lumicell, prior to surgery, the clinical site must update the subject in the EDC to document the subject's status as 'enrolled'. Once a subject is classified as 'enrolled', the subject will be randomized to either the control arm or the treatment arm of this study. Although the subject is randomized at the time of enrollment, the randomization will not be revealed to the study team until after the standard of care lumpectomy is completed.

5.2.4 Main specimen resection

The surgeon shall remove the main specimen per standard of care with the intention of achieving grossly negative margins. See Appendix A for guidelines on how to handle and ink the main specimen. Before the main specimen resection is complete, the LUM Imaging Device shall be initialized and the sterile barrier shall be applied according to the Instructions For Use. A Study Worksheet provided by Lumicell shall be completed by the clinical site throughout the surgery to collect study related information.

5.2.5 Lumicell cavity initialization

The Lumicell cavity initialization shall be performed by recording images of the lumpectomy cavity walls per Instructions For Use manual to set the tumor detection threshold specific for that patient.

5.2.6 Standard of care shaves

Before removing standard of care comprehensive shaves or selective shaves (based on palpation of the main specimen or cavity walls, X-ray imaging of the intact main specimen, intraoperative frozen section pathology, or visual examination), the surgeon shall record a LUM image from all the orientations from which an SOC shave will be removed. At this time, the tumor detection algorithm will be "off" to allow fluorescence visualization and navigation of the lumpectomy cavity without highlighting of suspected tumor that may bias normal standard of care.

5.2.6.1 Prior to obtaining any SOC shaves or associated images, the surgeon shall document their intent of obtaining SOC shaves on the study worksheet.

5.2.6.2 When there is no tissue available to be removed from a given orientation (e.g., too close to skin or too close to the chest wall), no LUM image shall be collected from that orientation. This information must be documented.

5.2.6.3 The surgeon shall place the optical head at the cavity location where a standard of care shave will be taken, instruct the computer operator when to save the image and define the image orientation.

5.2.6.4 The surgeon shall take the standard of care shave from the imaged location.

5.2.6.5 The surgeon shall repeat steps 5.2.6.1-5.2.6.4 for all cavity location(s) where a standard of care shave will be taken.

5.2.6.6 The resected tissue(s) shall be handled and named according to the Appendix B.

Standard of care margin assessment will be made by pathology on all the tissue removed during the standard of care procedure. If a positive margin is determined, this subject will be included in

the positive margin population. The intent of the standard of care lumpectomy procedure is to achieve negative margins.

5.2.7 Randomization revealed

After completion of standard of care, the study coordinator (or other authorized person) shall log-in to the EDC system to reveal whether the patient will receive Lumicell image-guided intervention. Randomization will be performed at a ratio of 10:1; for every 10 patients undergoing Lumicell imaging directed tissue resection after standard of care ("to proceed"), 1 patient shall complete surgery following standard of care alone ("not to proceed"). The randomization is intended to mitigate potential surgeon bias, and the 10:1 ratio was chosen based on prior clinical studies that supported imaging agent efficacy trials.

5.2.8 Lumicell procedure (only patients randomized to the intervention arm)

After the standard of care is completed for patients randomized "to proceed" with the Lumicell intervention, the surgeon will scan the tumor bed to collect images of the cavity walls and remove therapeutic shaves as indicated by the LUM decision software (refer to the Instructions For Use manual for details). The software will automatically turn on the tumor detection algorithm to allow visualization and navigation of the lumpectomy cavity with highlighting of suspected tumor by the tumor detection algorithm.

5.2.8.1 When there is no tissue available to be removed from a given orientation (e.g., too close to skin or too close to the chest wall), no LUM image shall be collected from that orientation. This information must be documented.

5.2.8.2 The surgeon shall scan the cavity and generate images from each orientation. Images should be generated and saved for both LUM positive and negative results.

5.2.8.3 The surgeon shall take a Lumicell shave from each imaged location indicated as "positive" by the LUM decision software.

5.2.8.4 A maximum of two (2) Lumicell shaves may be taken from any one orientation. Additional Lumicell shaves beyond 2 per orientation will constitute a protocol violation.

5.2.8.5 The resected tissue(s) shall be handled and named according to the Appendix B.

If a shave is not taken when indicated by the LUM Imaging System or a Lumicell image is not recorded as described above, a protocol deviation will be recorded with exception as described in section 5.3.1. If images are not saved in all orientations, a protocol deviation will be recorded unless there is no enough tissue left to take a shave (see 5.2.8.1). The reason for not taking a shave when indicated by the Lumicell system shall be recorded.

5.2.9 Pathology

Pathology shall report on the following criteria for each sample of breast tissue excised during the surgery (main specimen and shaves). It is particularly important that the pathologist reports the tumor distance(s) to the specimen margin(s) for each specimen excised during surgery. Reporting on tumor distances to final margins alone will not allow for determination of study endpoints.

- Type of resection (e.g. lump or shave)
- Tissue dimensions (LxWxH)
- Tissue Weight (g), if available

- Presence/absence of tumor (Invasive Lobular Carcinoma (ILC), Invasive Ductal Carcinoma (IDC), IDC with Ductal Carcinoma *in situ* (DCIS), DCIS only or any combination of these)
- tumor distance(s) to inked margin(s)
- tumor grade
- tumor size
- Other tissue type(s) present, including:
  - LCIS
  - Atypical ductal hyperplasia
  - Usual ductal hyperplasia
  - Inflammation
  - Fibroadipose only
  - Fibroadenoma
  - Papilloma
  - Dense Fibrous
  - Fibrocystic change
  - Apocrine metaplasia
  - Radial scar lesions
  - Biopsy site changes
  - Adenosis

The final margin assessment for a patient is based on the outermost surface. The pathologist will be blinded to the type of shave (Lumicell-guided or standard of care) when conducting the pathology evaluation.

5.2.9.1 Data collected from re-excision surgery

Margin assessment of the outermost resection surface from the initial surgery will be used to inform the surgeon whether a patient should receive re-excision surgery. Re-excision surgery data as well as pathology data from re-excision surgery will be collected. Data will be collected on patient-provider interaction and discussions when evaluating the recommendation for re-excision.

5.2.10 Follow-up

Subjects will be followed, and data collected as described in section 8.4.

5.2.11 Safety observations

All subjects will be observed to assess the safety of LUM015 with standard preoperative, intraoperative and postoperative monitoring after receipt of the LUM015 injection. The subject will have a final safety assessment at the first post-operative visit, where a blood draw will occur, and lab values evaluated for clinically significant values that differ from the subject's baseline values. All subjects will continue their enrollment and be followed in the study until their medical team determines that no further surgical intervention is required.

5.2.12 Other general procedures and injection of LUM015

For subjects with non-palpable tumors, an ultrasound, mammogram or MRI-guided localization procedure may be performed prior to or the same day as the surgery. Also, patients having a sentinel lymph node (SLN) mapping procedure may be injected with the radiotracer Technetium-

99 (Tc-99) per standard of care. LUM015 may be injected before or after any of these procedures, as long as the injection occurs 4 ± 2 hours prior to the first image recorded.

Prior to the surgery, the LUM Imaging System will be positioned in the operating room and an initialization procedure will be conducted to ensure that the performance parameters of the device are within defined specifications. If during the initialization procedures, the performance parameters are outside the defined specification, the user is prompted to discontinue use of the device. If the error cannot be fixed within a reasonable time in the operating room, these patients will receive standard of care treatment and will not be imaged with the LUM Imaging Device. These patients will be followed and monitored for safety the same way as those patients that undergo imaging with the LUM Imaging Device. The patients that are not imaged with the LUM Imaging Device will be included in the safety cohort and will count towards device failure rate.

To avoid excessive tissue resection based on LUM015 signal, surgeons are not to take more than 2 therapeutic shaves in a given orientation.

5.3 Lumpectomy specimen and cavity walls orientation nomenclature

Throughout this protocol, reference is made to the orientation of the main specimen, the SCMs and the lumpectomy cavity walls. When a lumpectomy is performed, there are typically 6 surfaces defined for orienting the main specimen and SCMs for final margin assessment. **Figure 5** shows a schematic representation of the nomenclature for the lumpectomy cavity margins for a right-sided breast lumpectomy, showing 5 orientations. The sixth orientation (not shown in the figure) is the "anterior" margin. Shaved cavity margins removed under the guidance of the LUM Imaging System will be named "therapeutic shaves" to distinguish them from standard of care SCMs. Please see Appendix B for the guidelines on naming the resected tissue. Excised tissue should be stitched and inked according to Appendix A.

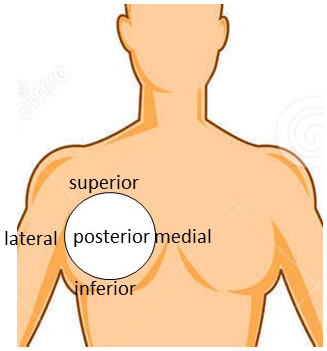


Figure 5: Schematic representation of the surfaces of the lumpectomy cavity walls (white circle). The posterior margin is nearest the fascia of the pectoral muscle. The anterior margin (not shown) is nearest the skin.

5.3.1 Guidance on removing therapeutic shaves

Therapeutic shaves should be removed anytime the LUM Imaging System indicates signal above the threshold (LUM positive signal shown in red in the computer screen). However, if there is not enough tissue to be removed (e.g. too close to skin, too close chest wall), a Lumicell image shall not be recorded and a therapeutic shave shall not be removed. This should be documented. Protocol deviations for not imaging and for not obtaining a therapeutic shave will not be issued when tissue is not obtained because there is not enough tissue to be removed.

5.3.2 Data collection

Clinical sites are expected to collect and enter data into the electronic case report form. Certified copies of applicable source documents are to be submitted to Lumicell, which include but are not limited to pathology reports for the lumpectomy and study procedure in addition to pathology reports for any re-excision surgeries. Additional source documents may be used to collect study-specific data on the day of surgery or during follow-up.

5.3.2.1 Patient Reported Outcome Measures (PROMs)

Survey data will be collected directly from the patient via an electronic survey tool. Patients who consent to the collection of PROMs will provide an email address at the time of consent. This email address will be entered into the electronic data capture system, but will be blinded to the Sponsor and Sponsor representatives and only available to database administrators and the clinical site. Surveys will be collected at four time points: (1) baseline (before lumpectomy), (2) standard of care follow-up after lumpectomy, (3) three months post lumpectomy and (4) six months post lumpectomy (± 6 weeks). Data to be collected may include information about the patient's breast satisfaction (cosmesis) and the patient's feelings about their breast cancer/breast cancer surgery ("cancer worry"). A validated survey tool developed by Memorial Sloan Kettering and McMaster University called the Breast-Q will be used to collect PROMs. Alternatives to electronic distribution of the surveys may be implemented.

5.3.3 Sentinel lymph node mapping procedures

Blue dyes commonly used for SLN mapping have fluorescence absorption and emission spectra that overlap with those from Cy5 (dye conjugated to LUM015). When blue dyes are used for SLN mapping, they are injected intra-tumoral or peri-tumoral soon after the patient is put under anesthesia and prior to the lumpectomy procedure. Therefore, patients planned to have injection of blue dyes for SLN mapping are excluded from this study.

As indicated in section 5.2.12, patients having SLN mapping procedures may be injected with the radiotracer Tc-99. In the event that not enough signal is produced from Tc-99, the surgeon may elect to inject a blue dye to assist in the mapping procedure. If injection of the blue dye happens before the LUM imaging procedure is completed, the subject will be excluded from the efficacy cohort but will remain in the safety analysis cohort.

5.3.4 Potential long-term data collection

Subject's participation in this pivotal study will be over after completing the planned follow-up in section 5.2.10. However, there may be additional information about the subject's cancer status that will be helpful to the study sponsors after the research study is complete. As part of participating in this research, the subject shall be asked to allow the researchers to access the patient's medical records for approximately five years after the initial breast cancer surgery. A separate IRB approval may be required to collect this data at a later time under a different protocol.

6 SUBJECT SELECTION

6.1 Eligibility Criteria

Subjects must meet the following criteria on screening examination to be eligible to participate in the study:

6.1.1 Subjects must have histologically or cytologically confirmed primary invasive breast cancer, ductal carcinoma in situ (DCIS) or primary invasive breast cancer with a DCIS component.

The methods for obtaining the histological samples can include core needle biopsies or fine needle biopsies. Subjects who had diagnostic open surgical biopsies are excluded from participation.

6.1.2 Female, age of 18 years or older. Because no dosing or adverse event data are currently available on the use of LUM015 in subjects <18 years of age, children are excluded from this study.

6.1.3 Subjects must be scheduled for a lumpectomy for a breast malignancy.

6.1.4 Subjects must be able and willing to follow study procedures and instructions.

6.1.5 Subjects must have received and signed an informed consent form.

6.1.6 Subjects must have no uncontrolled serious medical problems except for the diagnosis of breast cancer, as per the exclusion criteria listed below.

6.1.7 Subjects must have organ and marrow function within limits as defined below:

- Leukocytes $\geq$ 3,000/mcL
- Platelets $\geq$ 75,000/mcL
- total bilirubin within normal institutional limits
- AST (SGOT)/ALT (SGPT) $\leq$ 2.5 X institutional upper limit of normal
- Creatinine $\leq$ 1.5 mg/dL or creatinine clearance $\geq$ 60 mL/min/1.73 m^2 for subjects with creatinine levels above institutional normal.

6.1.8 Subjects with ECOG performance status of 0 or 1.

Note: Subjects with a history of multiple drug allergies, atopic subjects, and subjects with atopic syndrome are eligible for the study but should be pre-medicated according to institution standards prior to injection with the LUM015 imaging agent.

6.2 Exclusion Criteria

Subjects who exhibit any of the following conditions at screening will not be eligible for admission into the study.

6.2.1 Subjects who have been diagnosed with bilateral breast cancer and are undergoing a bilateral resection procedure.

6.2.2 Subjects who are pregnant at the time of diagnosis of their breast cancer; this exclusion is necessary because the teratogenic properties of LUM015 are unknown. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with LUM015, breastfeeding should be discontinued if the mother is treated with LUM015.

6.2.3 Subjects who are sexually active and not willing/able to use 2 medically acceptable forms of contraception (hormonal, barrier method of birth control, abstinence) upon entering the study and for 60 days after injection of LUM015. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Breast cancer patients are routinely advised against becoming pregnant during treatment, so this requirement does not differ from standard of care.

6.2.4 Subjects who have taken an investigational drug within 30 days of enrollment.

6.2.5 Subjects who will have administration of methylene blue or any dye for sentinel lymph node mapping on the day of the surgery prior to imaging the lumpectomy cavity with the LUM Imaging Device.

6.2.6 Subjects who have not recovered from adverse events due to other pharmaceutical or diagnostic agents.

6.2.7 Subjects with uncontrolled hypertension defined as persistent systolic blood pressure > 180 mm Hg, or diastolic blood pressure > 110 mm Hg; those subjects with known HTN should be stable with controlled HTN while under pharmaceutical therapy.

6.2.8 History of allergic reaction to polyethylene glycol (PEG).

6.2.9 History of allergic reaction to any oral or intravenous contrast agents.

6.2.10 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, COPD or asthma requiring hospitalization within the past 12 months, or psychiatric illness/social situations that would limit compliance with study requirements.

6.2.11 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with LUM015.

6.2.12 Any subject for whom the investigator feels participation is not in the best interest of the subject.

6.2.13 Subjects undergoing a second lumpectomy procedure because of positive margins in a previous surgery prior to entering this study.

6.2.14 Subjects with post-biopsy hematomas greater or equal to 2 cm that are visible on physical exam or detected during pre-operative observations.

6.2.15 Subjects with prior ipsilateral breast cancer surgeries, mastectomies, breast reconstructions or implants.

6.2.16 Subjects with prior ipsilateral reduction mammoplasties (breast reductions) performed less than 2 years prior to enrollment to this study.

6.2.17 Subjects previously treated with systemic therapies to treat the cancer to be removed during this clinical investigation, such as neo-adjuvant chemotherapy or hormonal therapy.

6.2.18 Subjects undergoing breast conserving surgery whose resected specimen (main lump, shaves, or any other resected tissue) will be evaluated with frozen section after the Lumicell-guided removal of shaves.

Note: It is unknown whether neoadjuvant radiation therapy affects the tumor environment and its response to LUM015; thus, patients previously treated with neoadjuvant therapy should be excluded per 6.2.17.

6.3 Inclusion of Women, Minorities and Other Underrepresented Populations

As this study is to test the efficacy of an intraoperative imaging technology in female breast cancer subjects, all of the subjects will be women. Males with breast cancer (<1% of breast cancer patients) usually undergo mastectomy procedures and only rarely have lumpectomies, and thus are not eligible for this study.

7 SUBJECT ENROLLMENT

7.1 General guidelines for screening and enrollment

Patients who have consented to participate in the study will be screened against the eligibility criteria. Patients who do not meet the inclusion/exclusion criteria listed in the protocol will fail screening and not be eligible to participate in the study. The subject is not considered enrolled into the study until written Informed Consent is obtained, the patient is confirmed as eligible per inclusion and exclusion criteria, and the investigational product has been administered (Study Day One).

Clinical sites are asked to keep comprehensive Screening and Enrollment logs and provide these logs to Lumicell on a regular basis.

7.2 Enrollment process

Patients who consent to participate in this study should be evaluated for eligibility. All patients who consent should be entered into the electronic data capture (EDC) system. Screening procedure data should be recorded in the EDC. The site study team should notify Lumicell of a potential candidate for the study and expected surgery date after the subject has been consented.

The enrollment procedures are as follows:

1. Obtain written informed consent from the subject prior to the performance of any study related procedures or assessments.
2. Assign the subject a study subject ID number and enter the subject in the EDC. The numbers should follow the Lumicell assigned format as described at the Site Initiation Visit in compliance with the Case Report Form nomenclature. All patients who consent should be entered into the electronic data capture (EDC) system.
3. Confirm protocol-specific eligibility using the eligibility assessment documented in the subject's medical/research record. **To be eligible for enrollment to the study, the subject must meet each inclusion and have none of the exclusion criteria listed in the eligibility section of this protocol.**
4. Notify Lumicell clinical team of eligible subject status and date of proposed surgery.
5. The subject is considered 'enrolled' once they have been administered LUM015 (day of surgery)

8 INTERVENTIONAL PLAN

8.1 LUM015 administration

The investigational imaging agent LUM015 will be administered at the surgical center or hospital 4 ± 2 hours prior to the first image recorded. See section 9 for more details about the preparation and administration of LUM015.

No investigational or commercial agents or therapies other than those described in this protocol may be administered with the intent to image the subject's malignancy intraoperatively.

As per IV injection standard of care, all subjects will be observed for signs of extravasation, or allergic reaction following administration of LUM015 to monitor for adverse pharmacological activity related to the investigational agent. If extravasation is suspected or confirmed, the injection

must be stopped immediately and reported to Lumicell. All study safety assessments will continue until the first post-operative visit (approximately 2-3 weeks after surgery).

If there is evidence supporting that extravasation of LUM015 injection occurred, then that patient will be excluded from the efficacy cohort. However, the patient will be included in the safety data analysis.

If the patient is experiencing an allergic reaction to LUM015 administration, the injection should be stopped immediately, and the following lab values should be obtained:

- Histamine
- Total Blood Complement
- Tryptase levels

The blood draw for each of these tests should be done as soon as possible after the start of the reaction and again at 30 minutes post start of the reaction. If an immediate blood draw is not possible, collect a 30-minute blood draw at minimum. Each blood specimen should be processed, packaged and shipped to the contracted central laboratory for this study. Specific instructions on collection, processing, and shipping can be found in the training materials associated with this study.

In case of an allergic reaction to LUM015, sites should not discard any residual drug remaining in the used syringe. This drug should be stored at -20 degrees C until a Lumicell representative notifies your site of how to proceed with the used syringe. If this process conflicts with site specific SOPs, the clinical site should notify Lumicell in advance of study start that residual drug will not be able to be maintained.

8.2 General concomitant medication and supportive care guidelines

A complete listing of all medications (including non-prescription, vitamins, herbal products and essential oils) currently being taken on a regular basis prior to surgery will be obtained at screening and updated as needed at enrollment and at the post-operative follow-up visit. Medications administered during the study related surgery should be recorded. Concomitant medications should be collected for up to 2 weeks after LUM015 injection and study related surgery. Concomitant medications should only be collected after this time point if a related AE/SAE is reported during the follow-up period. Concomitant medications will be recorded in the medical record and case report form.

8.3 Duration of therapy

The imaging agent LUM015 will be injected as a single dose 4 ± 2 hours prior to the first image recorded. There is no recurrent administration of the imaging agent. The treatment ends after the surgery is completed. Subjects may be discontinued by the principal investigator at any time for any of the following reasons:

- unacceptable adverse event or adverse device effect
- administrative reasons, such as imaging agent no longer available
- subject noncompliance,
- safety concern,
- subject decides to withdraw from the study, or

- general or specific changes in the subject's condition render the subject unacceptable for further treatment in the opinion of the treating investigator.

8.4 Duration of follow up

Subjects will continue their enrollment and be followed in the study until their medical team determines that no further surgical intervention is required and their post-operative follow-up and post-operative blood sample has been completed or until resolution of any reported adverse event related to the study intervention.

When attending their first post-operative visit a blood sample will be collected for a CBC with differentials (red blood cells, white blood cells including neutrophils, lymphocytes, monocytes, basophils, and eosinophils, and platelets) and serum chemistry (alkaline phosphatase, total bilirubin, BUN, calcium chloride, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium and creatinine/creatinine clearance) tests to assess for possible adverse events. At the time of the visit, the patient will be interviewed to determine any potential adverse events. Subjects with adverse events that are determined to be possibly related to the investigational product will be followed until resolution or stabilization of the adverse event. This visit occurs per the standard of care post-operative visit schedule; there is no protocol specified window. In the event a post-operative standard of care visit does not occur, the reason for no visit should be documented and the patient should be contacted by the research staff to be interviewed for adverse events.

For subjects who opt to participate, PROMs will be collected via electronic survey at four time points: (1) baseline (before lumpectomy), (2) standard of care follow-up after lumpectomy, (3) three months post lumpectomy and (4) six months post lumpectomy. Surveys will be sent to the study subject via a secure portal associated with the electronic data capture (EDC) system. Alternatives to electronic distribution of the surveys may be implemented. Patients who are participating in the completion of the PROMs survey data will not have new adverse events collected or reported after the follow-up period has concluded as described in this section.

8.5 Criteria for removal from the study

Subjects will be removed from study when any of the criteria listed in Section 6.2 applies. The reason for study removal and the date the subject was removed must be documented in the study-specific case report form (CRF). A subject removed from the study will be given standard of care treatment. Subjects removed from the study prior to dosing do not count toward total enrollment numbers; they are considered “screen failures”.

Individual patients may be discontinued from the study by the Investigator or Lumicell at any time if either determines that it is not in the best interest of the patient to continue (e.g., continuation in the study represents a serious medical risk to the patient). This may include, but is not limited to, the presence of serious, life-threatening adverse events, unanticipated adverse device effects, adverse events, or adverse device effects that are unacceptable in nature, severity, or frequency as assessed by the Investigator.

Patients must not be enrolled if, prior to study drug injection, they become pregnant or withdraw consent. While patients will be encouraged to complete the study, they may voluntarily withdraw at any time.

In the event of unusual or life-threatening complications, participating investigators must immediately notify Lumicell.

An individual is considered to be a participant once she is enrolled or entered into the study. This would include subjects that are eligible for contributing data to the scientific aims and consented for participation, including individuals who subsequently dropout. Subjects who are screened for participation but are not eligible (because of not meeting inclusion/exclusion or because LUM015 was not injected) or who do not consent would not be considered as participants.

Prospective exclusions from the efficacy analysis population will include: 1) patients for whom the LUM015 systemic injection was not performed properly and (2) major protocol deviations. These patients will be included in the safety analysis cohort.

8.6 Criteria for stopping the study

The events listed below are potential stopping rules criteria for the study. Any event listed below will be reviewed by the Data Safety Monitoring Board and may be deemed as a stopping criterion for the study. If the study is stopped, no additional patients will be recruited, dosed or imaged until the Data Safety Monitoring Board has approved the study to continue.

- Recommendation by the Data Safety Monitoring Board after evaluation of cumulative safety reviews
- Any death probably or definitely related to the treatment with LUM015 or the Imaging Device.
- Any other event that is deemed unacceptable in nature, severity, or frequency as assessed by the Data Safety Monitoring Board.
- Data Safety Monitoring Board recommendation after unexpected event

In addition to routine Data Safety Monitoring Board meetings, an ad-hoc meeting will occur if the occurrence of allergic reactions occurs more frequently than the expected rate. The DSMB will review details of the events and will generate a recommendation for study continuation or termination.

In the case that a safety event requiring enrollment suspension occurs, a prompt cumulative review of safety data and the circumstances of the event in question will be conducted to determine whether recruitment can be resumed, whether the protocol should be modified, or whether the study will be discontinued permanently. The FDA and reviewing IRB must be notified of any event that triggers suspension of enrollment in this study. If enrollment is suspended for safety reasons and it is deemed appropriate to resume the study, approval from the Data Safety Monitoring Board must be obtained prior to resuming the study. The FDA and reviewing IRB will be notified when enrollment in this study is resumed.

Regardless of whether recruitment is continued or not, all subjects injected with LUM015 or who were imaged with the LUM device at the time of study-stopping criteria were met will continue to be followed for safety.

In addition to the safety stopping rules outlined above, Lumicell may suspend or terminate this study at any time. The reasons for temporarily suspending or terminating the study may include but are not limited to the following:

- Subject enrollment is unsatisfactory
- Non-compliance that might significantly jeopardize the validity or integrity of the study.
- Lumicell decision to terminate development.

9 LUM015 FORMULATION AND ADMINISTRATION

Complete instructions for the preparation and administration of LUM015 can be found in the Lumicell Pharmacy Manual. A description of LUM015 including storage and stability information, handling, and administration in also found in Lumicell Pharmacy Manual and Investigator's Brochure.

9.1 Availability

LUM015 is an investigational agent and will be supplied free-of-charge from Lumicell, Inc.

9.2 Administration

LUM015 is administered over 3 minutes as a single dose of 1.0 mg/kg via peripheral intravenous (IV) injection in 0.45% Sodium Chloride Injection, USP, 4 ± 2 hours prior to the first image recorded. The IV line must be flushed with 10-20 mL of normal saline just prior to injection of LUM015 and the injection is immediately followed by a saline flush of 10-20mL. If extravasation is suspected or confirmed, the injection must be stopped immediately and reported to Lumicell.

Per Principal Investigator's discretion, in order to protect subjects from potential anaphylactic reaction, prophylactic treatment with diphenhydramine may be administered.

In case of an allergic reaction to LUM015, sites should not discard any residual drug remaining in the used syringe. The syringe/used drug should be stored at -20°C until a Lumicell representative notifies your site of how to proceed with the used syringe.

9.3 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a supply accountability record of the inventory and disposition of Investigational Product, including LUM015, camera covers (also known as sterile barriers), and the imaging device. Ancillary clinical supplies will be distributed. Receipt and return should be documented, when applicable.

9.4 Receipt and return of LUM015 and the LUM Imaging Devices

A drug inventory will be maintained by the clinical site's pharmacy. The inventory will include details of the LUM015 received and a clear record of when dispensed and for which subjects. This inventory record shall indicate the quantity and disposition of all investigational materials on hand at any time during the study.

LUM Imaging Devices will be assembled by Lumicell personnel and Instructions For Use (IFU) will be provided. Lumicell personnel will train the clinical staff on using the LUM Imaging System. Each clinical site investigator will maintain a device accountability record to document receipt and return of the LUM Imaging Devices.

At the end of the study, unused supplies of LUM015 and the investigational devices must be returned to Lumicell. A Lumicell representative will advise you when to return the supplies, as well as the proper shipping methods. Any LUM015 destroyed according to institutional policies will be documented. LUM015 should only be destroyed instead of returning to Lumicell if written instruction to do so is provided by Lumicell.

10 Schedule of Events

Baseline evaluations are to be conducted within 8-weeks prior to subject enrollment. The breast cancer diagnosis is not considered a baseline evaluation. All baseline assessments must be performed prior to administration of any study agent.

Table 2: Schedule of events.

	Pre-Enrollment /Screening	Day 1 / Enrollment	~2-14 days after surgery	Routine follow up visit	3-month PROM survey collection	6-month PROM survey collection
Informed consent	X					
Medical History	X					
Radiologic evaluation*	X					
Physical exam (Ht, Wt, VS)	X					
Pregnancy test (serum or urine)	X[b]					
CBC with differentials	X			X		
Serum chemistry [a]	X			X		
Concomitant Medications	X	X		X		
Adverse event/adverse device effect evaluation		X		X		
Patient Reported Outcome Measures Survey**		X**		X	X	X
LUM015 administration		X				
Randomization		X				
Intraoperative imaging***		X				
Margin assessment			X			

*: Radiologic Evaluations are not required if not part of the patient's medical history

**: PROs are optional for enrollment. The baseline evaluation can be completed by the subject at any time prior to the lumpectomy procedure. A validated survey tool, the Breast-Q, will be used to collect the majority of the PROMs.

***: If subject is randomized into the device arm

a: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium and creatinine/creatinine clearance.

b: Serum or urine pregnancy test (women of childbearing potential)

11 SAFETY

A Phase 1 IND study in sarcoma and breast cancer patients was completed at Duke University Medical Center. The primary endpoint of this study was to evaluate the safety of the LUM015 drug component. As a secondary endpoint, the signals from normal and tumor tissue were measured ex vivo using the LUM Imaging Device component. Twelve (12) sarcoma patients and three (3) breast cancer patients (invasive ductal carcinoma) were injected with LUM015 at doses ranging from 0.5 mg/kg to 1.5 mg/kg with no adverse pharmacological activity reported. The only noticeable effect in the study subjects was blue/green discoloration of urine, which resolved within 12-24 hours post injection for most patients.

Three feasibility studies have been completed in breast cancer with 294 patients enrolled (n=15 in Phase A, n=45 in Phase B, n=234 in Phase C), with 289 patients injected with LUM015. One patient enrolled in Phase B experienced blue discoloration at the injection site due to extravasation of LUM015 resulting from incorrect positioning of the intravenous catheter. After 92 days from injection, the blue discoloration disappeared but slight pallor of the skin at the injection site remained. One patient, with known hypersensitivity to contrast agents enrolled in the Phase C study experienced an anaphylaxis reaction to the LUM015 injection. The patient recovered in <24 hours.

11.1 LUM015 pharmacokinetics

During the Phase 1 IND study, the first 3 eligible subjects were dosed at 0.5mg/kg LUM015 and the second 3 subjects were dosed at 1.0mg/kg LUM015, with both groups injected ~29 hours prior to surgery. Resected tissue was imaged with the LUM Imaging Device in the pathology suite. A comparison of pharmacokinetic data from the initial 6 subjects to preclinical mouse studies suggested that a higher tumor:normal signal ratio would be achieved if tissue were imaged 4-6 hours after injection. The next 3 subjects were injected with 1.0mg/kg of LUM015 and followed by 3 subjects injected with 1.5 mg/kg of LUM015 and the final cohort of 3 subjects was injected with 0.5mg/kg. These nine subjects were followed by surgical resection at approximately 6 hours after LUM015 injection (i.e. same day injection and surgery).

To evaluate the pharmacokinetic parameters of LUM015, plasma PK samples were collected at the following time-points: pre-dose, 10, 20 minutes, 1, 2, 4, 8, 12, 18, 22, and 48 hours post-dose. The 48-hour collection was optional. Analysis of LUM015 (as Frag 1 after trypsin digestion of plasma) and Fragment 1 (non-digested plasma) were measured by LC-MS/MS. Additional metabolites, Fragment 2 and Fragment 3 were measured by HPLC-fluorescence.

A non-compartmental approach within Win-Nonlin software was used for PK parameter estimation. Due to a relative complexity of the PK profile observed and data constraints inherent in the study (small number of patients per dose level, optional data from 22-48 hours), a compartmental modeling approach was not utilized. Out of the 15 subjects, the 48-h time-point sample was collected in 9 patients. Thus, in order to make use of the wealth of data collected up to 22 hours (all 15 patients from the study), but also use potentially important 48-hour data from the limited set of 9 subjects, PK calculations were performed on both sets (22-hour and 48-hour) independently. A summary of the resulting PK parameters for LUM015 are presented in Table 3.

Table 3: Pharmacokinetic parameters for LUM015. SD = standard deviation.

PK Parameter	0.5 mg/kg (SD)	mg/kg (SD)	1.5 mg/kg (SD)

Tmax [hr]	0.11 (0.17)	0.06 (0.13)	0.00 (0.00)
Cmax [ug/mL]	15.46 (2.26)	28.41 (6.55)	45.76 (3.78)
AUClast [ug mL-1 h]	78.36 (12.91)	159.65 (45.94)	252.08 (57.98)
T1/2 [h]	5.21 (1.33)	5.08 (0.86)	5.42 (0.67)

The pharmacokinetic data support rapid clearance of LUM015. After 22 and 48 hours, only 5% and 2% of the parent drug remain in plasma, respectively. The PK data support a multi-phased profile with linear pharmacokinetics.

11.2 Expected adverse events (associated with LUM015)

During Phase C of the Feasibility Study, one patient had an anaphylactic reaction to the LUM015 injection. This patient had a history of allergic reactions to contrast agents. The event was resolved within 24 hours of occurrence. We now consider anaphylactic reaction an expected adverse event, with occasional occurrence and serious severity. Table 4 below lists the expected adverse events due to LUM015 injection with their estimated occurrence and severity.

Table 4: List of expected adverse events due to LUM015 administration.

Adverse Events (Drug Related):	Occurrence:	Severity:
LUM015 extravasation	Occasional	Minor
Chromaturia	Frequent	Negligible
Anaphylaxis/hypersensitivity	Occasional	Serious

No adverse events related to the administration of LUM015 were observed during the Phase 1 IND safety study. Temporary blue/green discoloration of the urine was noted in these patients due to the blue color of LUM015. Prior to the Phase 1 study, LUM015 had not previously been used in humans. Lumicell's preclinical studies demonstrated that it was reasonably safe to proceed with a Phase 1 IND study in humans. Preclinical studies in rats showed no LUM015 related effects on clinical observations, FOB evaluation, body weights, food consumption, ocular condition, clinical chemistry, hematology and coagulation parameters or organ weight at doses up to 53-fold higher than in humans.

The results from the repeat dose toxicity study in dogs (performed by NCI) show that administration of 0.5 or 10.0 mg/kg of LUM015 intravenously once daily for seven consecutive days (8 total doses) did not cause any observable target organ toxicities. The only observable effect was hypersensitivity in several dogs.

The results from a repeat dose toxicity study in rabbits show that administration of 15 mg/kg of LUM015 intravenously one daily for seven consecutive days was well tolerated. The test article-related findings were limited to transient blue/green discoloration of the urine. There were no test article-related adverse events observed and no hypersensitivity was seen.

Additional information is available in the Investigator's Brochure for LUM015.

Clinicians should be prepared for a possible hypersensitivity or allergic reaction to occur during each administration of LUM015. Standing orders should be in place in the event of a hypersensitivity or allergic reaction for immediate intervention including administration of

diphenhydramine, prednisone or both. If a study subject develops a Grade 3 (or greater) allergic reaction as defined in CTCEA 5.0, Lumicell should be contacted within 24 hours of the event:

To Lumicell:
Jorge Ferrer, Ph.D.
Phone: 617-571-0592
Email: jmferrer@lumicell.com
Fax: 781-672-2501

11.3 Expected adverse device effects (associated with the LUM Imaging System)

To date in the Phase 1 and Feasibility Studies, there have been no adverse device events associated with use of LUM Imaging Device. Patients in this trial will be monitored for potential infections, hematomas or other intraoperative or post-operative adverse device events. A list of potential adverse device effects is included in the Investigators Brochure.

12 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

12.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study. The severity of any possible AE observed will be classified per the grading established by the Common Terminology Criteria for Adverse Events (CTCAE) 5.0.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

12.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the subject and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm

requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- respite care

12.1.3 Adverse device effect (ADE)

An adverse device effect (ADE) is an adverse event which is at least possibly related to the device. ADEs are not considered serious adverse events.

12.1.4 Unanticipated adverse device effects (UADEs)

Unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety or any life-threatening problem or death cause by or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigation plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety or welfare of subjects.

12.1.5 Expectedness

Adverse events (AEs) and adverse device effects (ADEs) can be 'Expected' or 'Unexpected'.

12.1.5.1 Expected adverse event or adverse device effect

Expected AEs and ADEs are those that have been previously identified as resulting from administration of the agent or use of the device. For the purposes of this study, an AE or ADE is considered expected when it appears in the current AE/ADE list, the Investigator's Brochure, the Instructions For Use or is included in the informed consent document as a potential risk.

12.1.5.2 Unexpected adverse event or adverse device effect

For the purposes of this study, an AE or ADE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current AE/ADE list, the Investigator's Brochure, the Instructions For Use, or when it is not included in the informed consent document as a potential risk.

12.1.6 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE or ADE is clearly related to the study treatment.
- Probable – The AE or ADE is likely related to the study treatment.
- Possible – The AE or ADE may be related to the study treatment.
- Unlikely - The AE or ADE is doubtfully related to the study treatment.
- Unrelated - The AE or ADE is clearly NOT related to the study treatment.

Final determination of relatedness will be made by the Medical Monitor, who will integrate all data, including investigator's assessment of relatedness.

12.2 Procedures for recording and reporting safety

The principal investigator will assess the occurrence of AEs, SAEs, ADEs and UADEs at all subject evaluation time points during the study.

All AEs, SAEs, ADEs and UADEs whether reported by the subject, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the subject's medical record and on the appropriate study-specific case report forms.

For events related to the imaging agent, the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

12.3 Reporting requirements

The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator and/or IRB.

The Investigator will be responsible to report SAEs that occur at the Institution to the IRB in accordance with IRB requirements. It is the responsibility of the principal investigator to report serious adverse events to Lumicell and/or others as described below.

12.4 Reporting to Lumicell

12.4.1 Adverse Event Reporting

Non-serious adverse events should be captured in the Case Report Form and the Adverse Event Log.

All serious adverse events that occur after the initial dose of LUM015 Imaging Agent must be reported to Lumicell within 24 hours of becoming aware of the event. This includes events meeting the criteria outlined in the definitions section for SAEs. In addition to reporting the SAEs as outlined above, report to Lumicell adverse events that meet the following criteria within 24 hours of becoming aware of the event:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention

The principal investigator or delegated study team member must report each serious adverse event to Lumicell immediately after learning of the occurrence. In the event that the Investigator does not become aware of the serious adverse event immediately (e.g., subject sought treatment elsewhere), the principal investigator is to report the event immediately after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events in writing to Lumicell according to the protocol training guidelines and:

To Lumicell:
Jorge Ferrer, Ph.D.
Phone: 617-571-0592
Email: jmferrer@lumicell.com

Fax: 781-672-2501

The Case Report Form (CRF) for a Serious Adverse Event should be completed as soon as possible after notifying Lumicell of the event. Written notification to Lumicell is required as indicated above in addition to the completion of the SAE CRF.

12.4.2 Unanticipated Adverse Device Effects

All unanticipated adverse device effects (UADEs) that occur during the study must be reported to Lumicell using the provided SAE form.

The principal investigator must report each unanticipated adverse device effect to Lumicell as soon as possible, but in no event later than 10 working days after the Investigator learning of the occurrence. Investigators should document their initial knowledge of the event. Report unanticipated adverse device in writing to Lumicell according to protocol training guidelines and:

To Lumicell:
Jorge Ferrer, Ph.D.
Phone: 617-571-0592
Email: jmferrer@lumicell.com
Fax: 781-672-2501

The Case Report Form (CRF) for a UADE should be completed as soon as possible after notifying Lumicell of the event. Written notification to Lumicell is required as indicated above in addition to the completion of the SAE CRF.

Lumicell will immediately conduct an evaluation of a UADE and must report the results of the evaluation to FDA, all reviewing IRBs, and participating investigators within 10 working days after the sponsor first receives notice of the effect. This evaluation will determine any action needed as described in section 8.6 of this protocol.

12.4.3 Non-Serious Adverse Event and Adverse Device Effect Reporting

Non-serious adverse events and non-serious adverse device effects will be reported to Lumicell on the adverse events Case Report Forms.

12.5 **Reporting to the Institutional Review Board (IRB)**

The investigative site will report adverse events, serious adverse events, adverse device effects and unexpected adverse device effects directly to the IRB in accordance with the institution's or Central IRB policy.

12.6 **Reporting to the Food and Drug Administration (FDA)**

Lumicell or its agents will report to the FDA via their IDE as required in 21 CFR Parts 312 & 812 and as additionally described in this protocol.

12.7 **Reporting to Hospital Risk Management**

The principal investigator will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

12.8 **Monitoring of adverse events/adverse device effects and period of observation**

All adverse events and adverse device effects, both serious and non-serious, and deaths that are encountered from initiation of study intervention and throughout the study, should be followed to

their resolution, or until the principal investigator assesses them as stable, or the principal investigator determines the event to be irreversible, or the subject is lost to follow-up. The presence and resolution of AEs, ADEs, SAEs and UADEs (with dates) should be documented on the appropriate case report form and recorded in the subject's medical record to facilitate source data verification.

For some SAEs/UADEs, the investigator, Sponsor, or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE/UADE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Subjects should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The principal investigator should notify Lumicell and the IRB of any unanticipated death or adverse event occurring after a subject has discontinued or terminated study participation that may reasonably be related to the study.

12.9 **Protocol deviations**

Protocol deviations to ongoing studies are any unapproved changes in the study design and/or procedures that are within the Investigator's control and not in accordance with the IRB approved protocol. The Principal Investigator or designee will enter all protocol deviations on the Protocol Deviation Tracking Log provided by Lumicell. The log will be reviewed and updated as necessary by the site team. The site monitor (CRA) will review the log during their interim monitoring visit and obtain a copy of the log for Lumicell files. Additionally, the site should inform the IRB of record of the protocol deviation following the IRB's policy on reporting deviations.

13 DATA AND SAFETY MONITORING

13.1 **Data reporting**

13.1.1 Method

Lumicell will collect, manage, and monitor data for this study.

13.1.2 Data submission

It is the expectation that all CRFs will be completed in a contemporaneous manner to the time of study related procedures. Timely completion of the reports will allow Lumicell to monitor the study conduct and data in an effective manner.

13.2 **Safety review**

A Medical Monitor will review and monitor adverse events data from this trial. The Medical Monitor will be a medical doctor with experience in oncology and who has no direct relationship with the study. Information that raises any questions about subject safety will be addressed with the Principal Investigator and study team.

13.3 **Monitoring**

Lumicell will conduct a study assessment followed by a site initiation visit (either face to face, or via web) in which the site team will receive protocol specific training in addition to a review of all investigator responsibilities, and expectations of Lumicell. Throughout the conduct of the study Lumicell will conduct intermittent site visits to assure compliance with the protocol and all applicable regulations. During these visits, there will be a source data verification of critical data

points transcribed to the CRF. Data monitoring may also be conducted via central and remote monitoring. At study completion, Lumicell will conduct a study closure visit. This study visit will assure that all site regulatory documentation is present and updated; all data queries are resolved; investigator sign-off/approval of all applicable study data submitted to Lumicell has occurred; and all final disposition instructions from Lumicell have been delivered to the site investigator. Involvement in this study as a participating investigator implies acceptance of the approved protocol, the potential for audits or inspections, including source data verification, by representatives of the FDA, Lumicell (or their affiliates), the institutional review board, or their representatives. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practices (GCPs), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, accuracy, and adherence to protocol requirements.

13.4 **Data Safety Monitoring Board**

An independent Data Safety Monitoring Board (DSMB) will be established to review the safety of the LUM Imaging System and to review any SAEs that occur during the study. The DSMB will develop and follow a DSMB Charter. The DSMB will be composed of at least 2 oncology specialists (at least one of whom specializes in surgical oncology) and a statistician. The DSMB will be provided with all reports of adverse events including SAEs regardless of investigator causality assessments.

Following the initial meeting, DSMB meetings will occur on a periodic basis in accordance with the DSMB charter. The chairperson of the DSMB will also be immediately provided with the report of any SAE that is judged as possibly, probably, or definitely attributable to treatment with the investigational product. The charter of the DSMB will specify that this committee is charged with providing periodic reports to Lumicell that contain recommendations that include, but are not limited to, (a) continuation of the study, and (b) termination of the study.

14 REGULATORY CONSIDERATIONS

14.1 **Protocol review and amendments**

This protocol, the proposed informed consent and all forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be mutually agreed upon by Lumicell. Such changes must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. Lumicell will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

In addition, any modifications to the protocol, consent or case report forms will be submitted to the FDA.

14.2 **Informed consent**

All subjects must be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The formal consent of a subject, using the current IRB approved consent form, must be obtained before any study-related procedures are performed. The consent form must be signed and dated by the subject or the subject's legally authorized representative, and by the person obtaining the consent. The subject must be given 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.

14.3 **Ethics and Good Clinical Practice (GCP)**

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 11 – Electronic Records; Electronic Signatures
    - Title 21 Part 50 – Protection of Human Subjects
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
    - Title 21 Part 56 – Institutional Review Boards
    - Title 21 Part 812 – Investigational Device Exemptions
- State laws
- Institutional research policies and procedures
- Contractual agreements

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research subject. In such case, the deviation must be reported to the IRB according to the local reporting policy.

14.4 **Study documentation**

The investigator and all designees must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research subject. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

14.4.1 Study Documentation Practices

a) The investigator must maintain adequate records to enable the conduct of the study to be fully documented
b) All records, including electronic forms, must be filled out completely. Complete each space or blank. If there is no information to go into a space or blank, then use the symbol "N/A". If an entire section or page of a record is "N/A", it is acceptable to indicate this by drawing one line through the entire section/page and use "N/A" near that line. It is acceptable to check a box marked "N/A" to indicate that a section or an entire page is not applicable. The use of 'White-Out' or similar correction fluid is forbidden.

c) Use of highlighters on records is acceptable so long as the highlighter color does not obscure the underlying text if the record is copied.
d) Accuracy is required. Always verify entries are correct and consistent with other information.
e) Signatures are to be authentic.
f) Recorded dates are to be the actual dates in which the activities were recorded. Back-dating is forbidden.
g) If drinks or chemicals are spilled on original records, dry them off to the best of your ability, make an immediate photocopy and make a notation of the event on the copy. Retain the original record except in the event of contamination with a hazardous material.
h) All forms must be filled out using non-erasable pen and must be legible. The use of blue or black ink is preferred.
i) Errors must be crossed out with a single line, the correction inserted, and the change initialed and dated by the approved person making the correction. The reason for the correction must be stated.

14.5 Records retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines, institutional policies or contractual agreement between Lumicell and the participating institution.

15 STATISTICAL CONSIDERATIONS

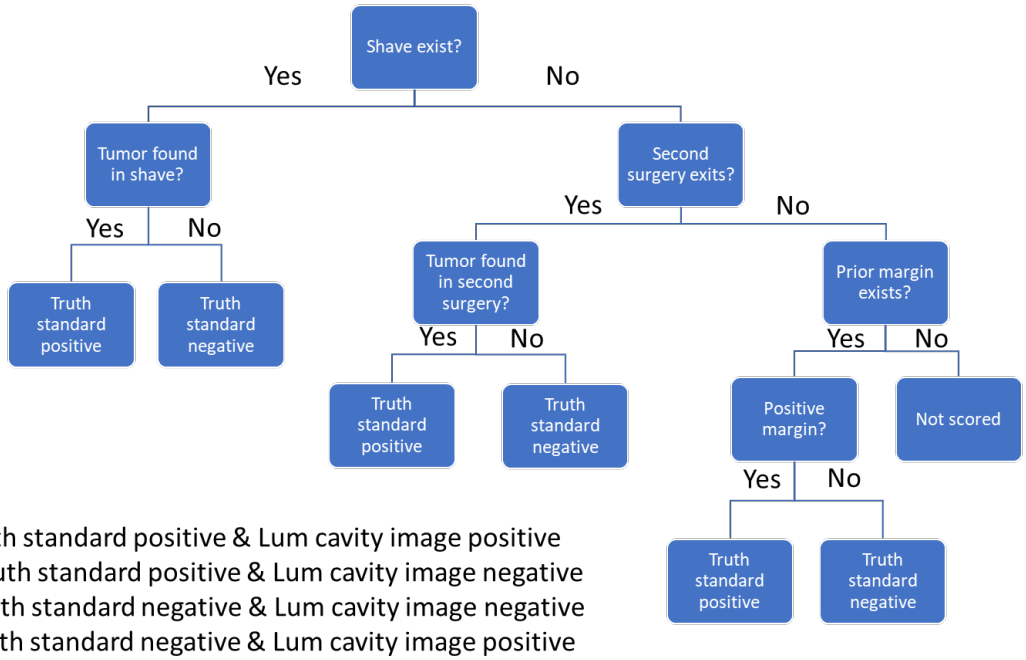
A full description of the data analysis is included in the Statistical Analysis Plan.

15.1 Analysis Populations

- Safety (SAF) Population: the safety population includes all subjects who received study drug (LUM015).
- Modified Intent To Treat (mITT) population: includes the subjects in SAF, but excluding subjects who are not able to be imaged with the LUM Imaging System. The mITT population will be used as the primary analysis population for the three co-primary efficacy endpoints and efficacy analysis of the secondary endpoints.
- Per-Protocol Population: Includes mITT subjects who complete all study evaluations and are without any major protocol deviations that may impact data collection or efficacy analysis. A sensitivity analysis of the primary and secondary efficacy endpoints will be conducted using the per-protocol analysis set.

15.2 Definition of truth standard for diagnostic performance evaluation (sensitivity and specificity)

We propose to use a hierarchical approach to determine the truth standard as therapeutic shaves were taken only when the LUM imaging system signaled positive for potential residual tumor. The highest truth standard is whether pathology finds cancer in a tissue shave removed from the area that was imaged in the cavity. When a shave does not exist, then whether tumor is found in a re-excision surgery is considered as the truth standard. When neither a shave nor a second surgery exists for a given orientation that was imaged, the margin assessment of that orientation is used as truth standard. **Error! Reference source not found.** shows a schematic for determination of the truth standard.



True positive = truth standard positive & Lum cavity image positive
False negative = truth standard positive & Lum cavity image negative
True negative = truth standard negative & Lum cavity image negative
False positive = truth standard negative & Lum cavity image positive

Figure 6: Definition of truth standard to evaluate the LUM Imaging System diagnostic performance.

When the therapeutic shave is not available and the follow-up surgery is performed, only the pathology from the first-time re-excision at the tissue/orientation level will be used for hierarchical sensitivity/specificity evaluation.

The orientation of the tissue removed during a re-excision is typically noted in the pathology report and matches the orientation in which a positive margin was identified from the initial surgery. However, there will be instances in which the orientation of the tissue removed during a re-excision is not specified. In the cases of unclear orientation of the tissue removed during the re-excisions, the orientations with positive margins will be used as positive/negative with cancer as suggested by the pathology of the resected tissue

If a mastectomy is performed as a follow-up surgery and no cancer found in the mastectomy specimen, all the orientation will be considered as cancer negative for truth standard. If mastectomy is performed and cancer is found and no indication of orientations where cancer has been found, the orientation in which a positive margin was identified in the initial lumpectomy will be considered as cancer positive for truth standard unless otherwise noted.

When a therapeutic shave and re-excision are not available for an orientation, the prior margin of the resected tissue of the corresponding orientation will be used as ground inferred truth for evaluation.

Refer to the associated Statistical Analysis Plan (SAP) for additional details.

15.3 **Sample Size**

The pivotal study will be powered based upon targeting success for the three co-primary endpoints. The efficacy endpoints will be assessed in the patients who undergo LUM imaging (not the 1/10th of subjects randomized to the control arm).

15.3.1 Residual cancer removal

The null hypothesis is that the true percentage of patients in whom at least one LUM-guided shave removed after the SOC procedure contain cancer as confirmed by pathology examination is smaller or equal than 3%:

$$H_0: p \leq 3\%$$

The alternative hypothesis is that the true percentage of patients in whom at least one LUM-guided shave removed after the SOC procedure contain cancer as confirmed by pathology examination is larger than 3%:

$$H_A: p > 3\%$$

where *p* is the true percentage of patients in whom at least one therapeutic shave removed after the SOC procedure contain cancer as confirmed by pathology examination.

The hypothesis will be tested using an exact Binomial test at a one-sided significance level of 2.5%. The true proportion p will be estimated along with a one-sided 97.5% exact confidence interval.

Lower bound selection for removal of residual cancer endpoint

To choose the success criteria for the proportion of patients who have residual cancer found in at least one LUM-guided shave, we used published results for estimates of local recurrence before and after adjuvant radiation, assuming that most local recurrences are a consequence of unresected cancer during the initial surgery. The meta-analysis conducted on approximately 28,000 patients to support the consensus guideline for margin in 2014 from SSO-ASTRO, reported an overall recurrence rate of 5.3% for patients undergoing lumpectomy and receiving whole breast radiation therapy (including patients with positive and negative margins) [1]. Other investigators have published local recurrence rates for patients with negative margins at 10.2% without a radiation boost, while 6.2% for those with radiation boosts [2]. Based on this data, we estimate that radiation addresses approximately 50% of residual cancer, but not all. Our Phase C results shows a proportion of 26/230 or 11.3% [95% CI: 7.5%-16.1%], supporting the lower bound selection of this endpoint.

Sample size for residual cancer removal

Our preliminary analysis of the Phase C exploratory data indicated that LUM detected residual cavity cancer in 11% of patients (26/230). To power the study, we are using a slightly more conservative assumption of a ~9% true percentage of patients in whom at least one therapeutic shave has cancer confirmed by pathology. With this assumption, enrolling 220 evaluable subjects, a binomial exact test for proportions at a 1-sided significance level of 2.5%, will have 90% power to declare the percentage of patients in whom at least one therapeutic shave has cancer confirmed by pathology to be larger than 3%.

15.3.2 Sensitivity and specificity

The verification of the sensitivity and specificity co-primary endpoints will be a refutation of the null hypotheses

$H_{01}^{s}: sen \leq 40\%$ against the alternative $H_{11}^{s}: sen > 40\%$

$H_{02}^{s}: spec \leq 60\%$ against the alternative $H_{12}^{s}: spec > 60\%$

where sen and $spec$ represent the true sensitivity and specificity, respectively. These hypotheses will be tested using an exact binomial test for testing proportions in those with a positive ground truth result (sensitivity) and those with a negative ground truth result.

Lower bound selection for sensitivity and specificity endpoints

Data from the Phase C Feasibility Study showed pathology margin assessment sensitivity to predict cancer in the cavity of ~40%. However, this pathology assessment is done several days after the surgery and can lead to second surgeries. Because the LUM Imaging System provides the additional benefit of assessing the cavity in real-time, a lower bound for instrument sensitivity > 40% is proposed as the success criterion for this hypothesis-tested endpoint. We expect the sensitivity to be similar to that observed in the Phase C study of 67% [95% CI: 56%-79%].

The Phase C data shows that at the current specificity performance of 70% [95% CI: 68%-73%], an average of 1.1 additional shaves were removed guided by the LUM Imaging system, accounting for ~10% of all the total resected tissue. We selected a lower bound for specificity of 60% to ensure that similar performance in the pivotal trial is obtain regarding tissue removal. Also, with this lower bound, a Youden Index > 0 will be demonstrated to indicate non-random diagnostic performance.

Sample size for sensitivity

Assuming a sensitivity of 60%, obtaining 70 truth standard positive events, a binomial exact test for proportions at a 1-sided significance level of 2.5%, will have 90% power to declare the lower bound of sensitivity > 40%.

Sample size for specificity

Assuming a specificity of 65%, obtaining 1021 truth standard negative events, a binomial exact test for proportions at a 1-sided significance level of 2.5%, will have 90% power to declare the lower bound of specificity > 60%.

15.3.3 Study sample size, event-driven design, and overall study power

The pivotal study is powered based upon targeting success for the three co-primary endpoints as outlined above. The efficacy endpoints will be assessed in the patients who undergo LUM-guided imaging (not the $1/10^{th}$ of subjects randomized to the control arm).

Based on Phase C data, it is expected that 268 patients will provide the necessary 70 truth standard positive events to power for sensitivity. Also, based on Phase C data, it is expected that 152 patients will provide the necessary 1021truth standard negative events to power for specificity. To power for the removal of residual cancer endpoint, approximately 220 patients are required. Thus, the size of this study is driven by the necessary number of truth standard positive events.

Adding 15% to the sample size of 268 patients to include subjects in the control arm and some expected data loss, it is estimated that 310 total patients will be needed for the pivotal study. However, given the uncertainty of translating the number of truth standard positive events to actual number of patients, the pivotal trial is planned as an event-driven clinical study. Patients will be enrolled until 70 truth standard positive events are reported, up to a maximum of 350 patients. The number of truth standard positive events will be counted based on the truth standard hierarchical approach from Figure 6. Counting of these events will be conducted via software programming to read from the electronic data capture system; no endpoints will be calculated at that time. Results

from the event counting process will be accessible to the DSMB which will then inform Lumicell personnel on whether the targeted number of events has been reached or if enrollment needs to continue.

At this sample size, the power for the sensitivity endpoint is 90%, for specificity is 99% and for removal of residual cancer is 96%. Thus, the overall study power is 90% x 99% x 96% = 86%.

Revision note: As of the release of revision 08 of study protocol CL0007, an event count conducted in April 1st, 2021 indicated that 380 patients need to be enrolled to achieve the necessary 70 truth standard positive events. We now intend to enroll up to **450** subjects in this study.

15.3.4 Overall Primary Hypotheses

The overall primary hypotheses for the primary endpoints is

$$H_0^p \text{ (null)}: H_0 \text{or } H_{01}^s \text{ or } H_{02}^s \text{ versus } H_1^p \text{ (alternative)}: H_1 \text{ and } H_{11}^s \text{ and } H_{12}^s$$

The removal of residual cancer endpoint will be tested at a one-sided significance level of 2.5%. Sensitivity and specificity endpoints will be tested at a two-sided significance level of 5%.

15.4 Statistical Methods

15.4.1 General considerations

All analyses will be performed under Good Clinical Practice (GCP) standards using a prespecified statistical analysis plan (SAP). All analyses will be conducted and tables and listings generated using Statistical Analysis System (SAS®) Version 9.4 or higher. Graphics may be prepared with SAS Version 9.4 or higher; R version 3.1.2 or higher (R Core Team (2014)), SigmaPlot 12.3 or higher (Systat Software, Inc., San Jose, California).

15.4.2 Demographic and baseline characteristics

Demographic and baseline characteristic including: age, race and ethnicity, menopausal status, mammographic breast density, palpability, cancer histologic type, tumor size, node positive patients, and tumor receptor status (ER/PR/HER2), and baseline body mass index (BMI) calculated from weight and height will be summarized for the study sample. Statistics for continuous variables will include mean, median, standard deviation (SD), minimum, maximum and sample size for the overall sample and for subgroups, and two-sided 95% CI of the mean difference between (control and study arms) and subgroups. Binary variables will be described with frequencies and percentages for the overall sample. Demographic and baseline characteristics will be summarized by overall using descriptive statistics for analysis populations.

15.4.3 Efficacy analyses

Three co-primary endpoints

- Ratio of patients who have **residual cancer** found in at least one Lumicell-guided shave (also known as therapeutic shave) among all patients randomized to the device arm.
- Diagnostic performance of instruments measured two separate endpoints as **sensitivity** and **specificity** in a per-tissue basis.

Secondary endpoints

a. Proportion of patients with positive margins after standard of care breast-conserving surgery who have a LUM Imaging System signal in the cavity above the threshold as defined by the tumor detection algorithm (i.e., a positive LUM signal).
b. Proportion of subjects with pathology-positive margins after standard of care breast-conserving surgery for whom additional LUM Imaging System-guided shaves resulted in pathology-negative margins among patients with positive margins. Note: This endpoint also estimates the potential reduction in re-excision surgeries following standard of care breast-conserving surgery that resulted from a positive SOC margin.
c. Proportion of subjects with pathology-positive margins after standard of care breast-conserving surgery for whom additional LUM Imaging System-guided shaves resulted in pathology-negative margins among all patients.
d. Ratio of patients with negative margins after the SOC procedure who have residual cancer found in at least one Lumicell-guided (or therapeutic) shave among patients with negative margins.
e. Ratio of patients with negative margins after the SOC procedure who have residual cancer found in at least one Lumicell-guided (or therapeutic) shave among all patients
f. Mean and median incremental volume (in cubic centimeters) of tissue removed from therapeutic shaves: For each patient, absolute shave volume will be calculated as length (c) × width (c) × depth (c) for each therapeutic shave with the sum by patient of all therapeutic shaves. Subjects with no therapeutic shaves taken will have a therapeutic shave volume of 0cc.
g. Mean and median contribution of therapeutic shave volume to total tissue removed.
h. Comparison of adverse events in subjects that have at least one therapeutic shave versus subjects that have no therapeutic shaves.
i. Average number of therapeutic shaves taken per subject overall and by type of SOC lumpectomy procedure (i.e., lumpectomy with comprehensive shaves vs. lumpectomy with or without selective shaves).
j. Percent of device failures that led to failure of using the LUM Imaging System to capture data.
k. Rate of re-excision procedures as a result of a positive margin status following SOC (control arm) and LUM Imaging procedures (device arm).
l. Number of re-excisions for each patient
m. Number of re-excisions recommended
n. Collect exploratory data on tissue types found in therapeutic shaves.
o. Collect exploratory data on patient reported outcomes.
p. Adverse events stratified by severity and relatedness to drug/device.
q. Serious adverse events stratified by severity and relatedness to drug/device.
r. Adverse events by preferred term (sorted by descending occurrences for each preferred term).
s. Summary of adverse events, overall and split by expectedness to drug/device

15.4.4 Safety analyses

Safety data will be reported as:

- Adverse events stratified by severity and relatedness to drug/device.
- Serious adverse events stratified by severity and relatedness to drug/device.

- Adverse events coded per MedDRA guidelines
- Adverse events by preferred term per MedDRA (sorted by descending occurrences for each preferred term).

All adverse events will be evaluated and assigned the "expectedness" by a Lumicell representative as described in the study plans or protocols and will be reported accordingly.

Two safety analyses sets will be completed: one for all the patients injected with LUM015 across all the Lumicell clinical studies (see **Error! Reference source not found.** in the Statistical Analysis Plan document), and another one for just the breast cancer patients.

15.4.5 Exploratory Data Analyses

Patient reported outcome measures (PROMs) will be reported as an exploratory endpoint. Also, the type of tissue found in therapeutic shaves will be reported as an exploratory endpoint.

In this protocol, the definition of positive margins is described in section **Error! Reference source not found.**. In addition, Lumicell will collect margin distance information such that alternative definitions (e.g. DICS on ink) can be tested in an exploratory manner.

15.4.6 Missing or spurious data

During Lumicell-guided imaging, when there is not enough tissue to be removed (e.g. too close to skin or too close to the chest wall) no LUM image shall be recorded because it does not generate an actionable result.

In the case where the LUM Imaging System indicates that a therapeutic shave needs to be removed but the surgeon decides not to follow this guidance, the reason for not doing so will be recorded in the patient's CRF or during imaging. These instances are considered as a protocol deviation. There are other cases when the shavings reach protocol limits so no shaving will be performed. In these cases, the data will be included in the performance evaluation as is - that is no Lum-guided shave-taken will be considered as no removing of cancer or positive margin. However, they will be considered as a detection if there are cancer in the re-excision or if there are positive margins on the corresponding orientations.

In occasions, a cavity image may be recorded from an unintended region or the image may be deemed as low quality (blurry, out of focus, etc.). For example, the surgeon realizes that he/she placed the device in the wrong location within the cavity after recording an image. Since the LUM software does not allow to overwrite saved images, this situation will be documented, the image will be excluded from the analysis.

16 PUBLICATION PLAN

The results of this study will be made public within 12 months of the completion of the study. Reference the Clinical Trial Agreement for detailed publication policy information.

17 REFERENCES

1. Moran, M.S., et al., *Society of Surgical Oncology-American Society for Radiation Oncology consensus guideline on margins for breast-conserving surgery with whole-breast irradiation in stages I and II invasive breast cancer.* Int J Radiat Oncol Biol Phys, 2014. **88**(3): p. 553-64.

2. Morrow, M., et al., *Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Ductal Carcinoma In Situ.* J Clin Oncol, 2016.
3. Kreike, B., et al., *Continuing risk of ipsilateral breast relapse after breast-conserving therapy at long-term follow-up.* Int J Radiat Oncol Biol Phys, 2008. **71**(4): p. 1014-21.
4. Singletary, S.E., *Surgical margins in patients with early-stage breast cancer treated with breast conservation therapy.* Am J Surg, 2002. **184**(5): p. 383-93.
5. Smitt, M.C., et al., *Predictors of reexcision findings and recurrence after breast conservation.* Int J Radiat Oncol Biol Phys, 2003. **57**(4): p. 979-85.
6. Dillon, M.F., et al., *A pathologic assessment of adequate margin status in breast-conserving therapy.* Ann Surg Oncol, 2006. **13**(3): p. 333-9.
7. Kurniawan, E.D., et al., *Predictors of surgical margin status in breast-conserving surgery within a breast screening program.* Ann Surg Oncol, 2008. **15**(9): p. 2542-9.
8. Schiller, D.E., et al., *Factors associated with negative margins of lumpectomy specimen: potential use in selecting patients for intraoperative radiotherapy.* Ann Surg Oncol, 2008. **15**(3): p. 833-42.
9. Smitt, M.C. and K. Horst, *Association of clinical and pathologic variables with lumpectomy surgical margin status after preoperative diagnosis or excisional biopsy of invasive breast cancer.* Ann Surg Oncol, 2007. **14**(3): p. 1040-4.
10. Waljee, J.F., et al., *Predictors of re-excision among women undergoing breast-conserving surgery for cancer.* Ann Surg Oncol, 2008. **15**(5): p. 1297-303.
11. Available from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/breast-cancer-facts-and-figures-2017-2018.pdf.
12. Chagpar, A.B., *Cavity Shave Margins in Breast Cancer.* N Engl J Med, 2015. **373**(22): p. 2187-8.
13. Coopey, S.B., et al., *Lumpectomy cavity shaved margins do not impact re-excision rates in breast cancer patients.* Ann Surg Oncol, 2011. **18**(11): p. 3036-40.
14. Weissleder, R. and U. Mahmood, *Molecular imaging.* Radiology, 2001. **219**(2): p. 316-33.
15. Mohamed, M.M. and B.F. Sloane, *Cysteine cathepsins: multifunctional enzymes in cancer.* Nat Rev Cancer, 2006. **6**(10): p. 764-75.
16. Gocheva, V. and J.A. Joyce, *Cysteine cathepsins and the cutting edge of cancer invasion.* Cell Cycle, 2007. **6**(1): p. 60-4.
17. Joyce, J.A., et al., *Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis.* Cancer Cell, 2004. **5**(5): p. 443-53.
18. Gocheva, V., et al., *IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion.* Genes Dev, 2010. **24**(3): p. 241-55.
19. Jaffer, F.A., et al., *Optical visualization of cathepsin K activity in atherosclerosis with a novel, protease-activatable fluorescence sensor.* Circulation, 2007. **115**(17): p. 2292-8.
20. Chen, B. and M.O. Platt, *Multiplex Zymography Captures Stage-specific Activity Profiles of Cathepsins K, L, and S in Human Breast, Lung, and Cervical Cancer.* J Transl Med, 2011. **9**: p. 109.
21. Lecaille, F., J. Kaleta, and D. Bromme, *Human and parasitic papain-like cysteine proteases: their role in physiology and pathology and recent developments in inhibitor design.* Chem Rev, 2002. **102**(12): p. 4459-88.

22. Naidu, R., et al., *Expression of c-erbB3 protein in primary breast carcinomas.* Br J Cancer, 1998. **78**(10): p. 1385-90.
23. Thomssen, C., et al., *Prognostic value of the cysteine proteases cathepsins B and cathepsin L in human breast cancer.* Clin Cancer Res, 1995. **1**(7): p. 741-6.
24. Lerebours, F., I. Bieche, and R. Lidereau, *Update on inflammatory breast cancer.* Breast Cancer Res, 2005. **7**(2): p. 52-8.
25. Nouh, M.A., et al., *Cathepsin B: a potential prognostic marker for inflammatory breast cancer.* J Transl Med, 2011. **9**: p. 1.

18 APPENDICES

Appendix A: Method for removing, orienting, inking and naming the main specimen and shaved cavity margins.

The methods described below are adapted from Cabioglu, et al. (ASO 14:1458-1471; 2007). This method applies to both palpable and non-palpable breast masses.

As described below, stitching of the main specimen (#2) and protocol specified nomenclature of the resected specimen (Appendix B) are protocol requirements to support data integrity. Non-compliance with these elements will be considered protocol deviations. Inking of the specimen and shaves, and stitching of the shaves are recommended per protocol but non-compliance is not considered a protocol deviation if the process negatively interferes with the institution's standard procedure.

Procedure:

1. Surgeon performs surgery attempting to obtain grossly negative margins in the main specimen.
2. Surgeon places orientation stitches at the superior and lateral orientations of the main specimen immediately after it is removed from the patient. **It is extremely important to maintain the orientation of the specimen relative to the lumpectomy cavity to be able to correlate LUM images of the cavity with pathology examination of the main specimen**.
3. Surgeon inks all surfaces of the main specimen using a differently colored ink for each orientation. Lumicell will provide an inking kit to facilitate this process. This kit is not part of the LUM Imaging System.
4. Surgeon may elect to remove additional tissue shaves based on palpation of the specimen or lumpectomy cavity walls, x-ray imaging of the main specimen (mainly for non-palpable masses), visual inspection, intraoperative frozen sections, standard of care shaves, or comprehensive shaves. If additional shaves are removed, the shaves must be labeled according to the orientation it was removed from and the number of additional tissue shaves taken from that orientation (see Appendix B for naming convention of resected tissue). For example, the first additional tissue from the medial orientation should be named as "medial 1".
5. If additional shaves are removed, the surgeon should ink as many surfaces as possible maintaining the same color used for a given orientation in the main specimen. For example, if the superior orientation of the main specimen is inked yellow, then the margin side of a tissue from the superior orientation should be inked yellow.
6. When any additional shaves are removed, the margin side should be marked with a stitch and should be inked per institution's standard procedure.

Appendix B: Guidelines for naming of resected tissues

Two (2) standard naming conventions, described as "Naming Convention B1" and "Naming Convention B2" in Table B below, shall be used by all sites to blind pathologists to whether the shave was excised during standard of care or Lumicell guidance. As the shaves are removed during the surgery, a record will be created in the operating room by the study personnel to indicate whether the tissue was excised during standard of care or Lumicell guidance (according to column B1). The pathologists must not have access to the record using naming convention B1 until after the pathology assessment has been recorded. The samples shall be labeled according to Naming Convention B2 before transfer from the operating room to pathology, and this naming convention shall follow the samples through the final pathology report.

Table B: Guidelines for naming resected tissues

Tissue	Naming convention B1 **Unblinded source for specimen orientation, shave number, and type of shave**	Naming example for surgical record and study source document (B1)	Naming convention B2 **Blinded naming convention for pathology**	Naming example for specimen to be sent to pathology (B2)
Main specimen	Main specimen [side of breast]	Main specimen, left breast	Main specimen [side of breast]	Main specimen, left breast
Standard of care shaves	[Orientation] [sequential shave number for an orientation] SOC	Superior 1 SOC	[Orientation] [sequential shave number for an orientation]	Superior 1
Therapeutic shaves	[Orientation] [sequential shave number for an orientation] Therapeutic	Medial 1 Therapeutic Medial 2 Therapeutic Superior 2 Therapeutic*	[Orientation] [sequential shave number for an orientation]	Medial 1 Medial 2 Superior 2 *

*Note this shave is named as "2" because a SOC shave was obtained in the same orientation. This allows for final margin status to be known when the specimen is blinded to type of shave for pathology evaluation.

Appendix C: Handling and sectioning SOC and therapeutic shaves

All cavity shaves, whether an SOC or therapeutic, will be handled and processed in the same manner as described below.

1. Section the shave in its entirety perpendicular to its long axis at approximately every 2 mm.
2. Place each section of a shave in a cassette for paraffin embedding. Note: if the sections are small enough, more than one section can be placed in a single cassette.
3. Prepare a paraffin embedded block from each cassette (e.g., if there are three cassettes for a single shave, this should result in three different blocks).
4. Prepare at least one histopathology slide for margin assessment from every single block.
5. Determine the margin status for each histopathology slide from a shave and record results.
6. Report results using naming convention B2, as described in Appendix B.

Example: 6 total shaves are removed from a patient and sectioning of the shaves generates 3 blocks each. The total number of slides for margin assessment would be: 6 shaves x 3 blocks/shave = 18 total slides from shaves.

Appendix D: Confidentiality and Confirmation Signatures (Protocol Signature Page)

Confidentiality agreement:

The Principal Investigator agrees to handle all information and documentation received from Lumicell, Inc. under the terms of the study agreement as well as the work performed and the results obtained during the duration and after termination of the agreement confidentially. Accordingly, all separate publications and lectures with any reference to the object of this agreement need the previous written consent of Lumicell, Inc. The evaluator ensures that all other persons involved in this project will maintain confidentiality as well.

Confirmation of the Principal Investigator:

Herewith I/we confirm to have understood and accepted all elements of the study protocol and the experimental part as agreed upon.

Location/Institution	Date
Signature of Principal Investigator (PI)	Printed name of PI