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Title: FEASIBILITY OF THE LUM IMAGING SYSTEM FOR INTRAOPERATIVE DETECTION OF RESIDUAL CANCER IN THE TUMOR BED OF FEMALE SUBJECTS WITH BREAST CANCER.
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IDE # G140195
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1. OBJECTIVES

1.1 Study Design

The objective of this non-randomized, open label study is to evaluate the safety and efficacy of an intraoperative imaging system, the LUM Imaging System (LUM015 in conjunction with imaging device LUM 2.6), in identifying residual cancer in the tumor bed of female breast cancer subjects.

The study is composed of a Feasibility Trial divided into Phase A (up to 15 total subjects, completed as of December 4, 2015) and Phase B (up to 50 total subjects). Phase A included 5 subjects not injected with LUM015 for autofluorescence tissue assessment and 10 subjects receiving LUM015 (as outlined below) to assess the ability to distinguish LUM015 from autofluorescence. Phase A concluded with the analysis of the data to (1) assess patient safety, (2) determine the parameters of the detection algorithm, (3) select the dose for Phase B and (4) evaluate the device function. These results were reported to FDA prior to starting the Phase B part of this study. In Phase A, no adverse events related to the investigational product were reported (n=15 patients). Lumicell also determined the tumor detection threshold parameters based on comparison of imaging data with pathology results. The dose selected for Phase B is 1.0 mg/kg based on Lumicell's dose-response model and Phase A data. During Phase B, we will preliminarily assess the performance of the detection algorithm and adjust the threshold parameters if needed.

Nomenclature:

Throughout this protocol, reference is made to the orientation of the lumpectomy cavity (tumor bed) and the shaved margins specimens. When a lumpectomy is performed, there are typically 6 surfaces defined as if the cavity is a cube. To determine the margin status (either positive or negative), a cavity shaving is taken from each surface of the tumor bed and analyzed by a pathologist. Note that there will be no intraoperative frozen section analysis of the gross resection or shavings during this study because frozen section analysis is not standard of care for breast cancer surgeries. **Figure 1** shows a schematic representation of the nomenclature for the lumpectomy cavity margins, showing 5 locations. The sixth surface (not shown in the figure) is the "anterior" margin which is the margin nearest to the skin which could blend into one or more of the side surfaces depending on how the initial incision is done. Typically, a shaved margin specimen is not taken from the posterior surface if the lumpectomy cavity reaches the fascia over the pectoral muscle. Thus, in a given lumpectomy, there will be between 4 to 6 shaved margin specimens.

Margins will be assessed per standard of care by a pathologist by measuring the distance to the inked surface of each tissue shaving from invasive cancer or ductal carcinoma in situ (DCIS). In this study, a positive margin is defined as invasive cancer or DCIS present on the inked surface.

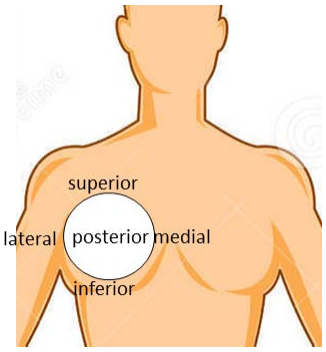


Figure 1: Schematic representation of the surfaces of a lumpectomy cavity (white circle). The posterior margin is nearest the fascia. The anterior margin (not shown) is nearest the skin.

For each of the lumpectomy surfaces, we use the naming convention shown in **Figure 2** to compare imaging versus pathology. For example, consider the lateral location. The surface of the gross resection facing the lateral location is called margin 1 (M1). The exposed surface in the lateral location of the lumpectomy cavity is called tumor bed 1 (TB1). Once the standard of care cavity shaving specimen is taken, the opposite side of the shaving is called margin 2 (M2). The newly exposed surface in the lumpectomy cavity is called tumor bed 2 (TB2). We use this naming convention throughout this protocol.

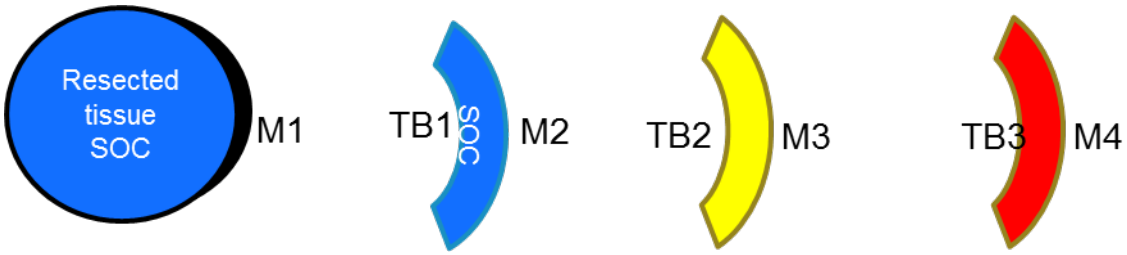


Figure 2: Schematic representation of the gross resection and the cavity shavings. Standard of care lumpectomy is depicted in blue (lumpectomy specimen and first cavity shaving). The second (yellow) and third (red) cavity shavings are only removed when the LUM Imaging System indicates that residual cancer is present (Phase B of the Feasibility study).

The following will be used as the definitions for determining the performance of the LUM imaging system at the per-tissue level.

- True positive = fluorescence above normal tissue threshold found in the sample + abnormal tissue found in the sample by histopathology
- False positive = fluorescence above normal tissue threshold found in the sample + no abnormal tissue found in the sample by histopathology
- True negative = no fluorescence above normal tissue threshold found in the sample + no abnormal tissue found in the sample by histopathology
- False negative = no fluorescence above normal tissue threshold found in the sample + abnormal tissue found in the sample by histopathology

<u>The Feasibility Trial</u>:

Phase A will consist of **15 evaluable subjects**

- Arm 1: 5 subjects not injected with LUM015
- Arm 2: 5 subjects injected with 0.5 mg/kg of LUM015
- Arm 3: 5 subjects injected with 1.0 mg/kg of LUM015

The first group of 5 subjects was not injected with LUM015 to measure the normal tissue autofluorescence baseline. The second group of 5 subjects was injected with LUM015 at a dose of 0.5 mg/kg. The third group of 5 subjects was injected with LUM015 at a dose of 1.0 mg/kg to assess the ability to distinguish LUM015 fluorescence signal from autofluorescence baseline. The objective was to determine the average tumor-to-normal tissue signal ratio (T:N) at both the 0.5 mg/kg and 1.0 mg/kg doses. In previous versions of the Investigational Protocol, the dose that generated the highest tumor-to-normal tissue signal ratio was going to be selected for Phase B. If both doses generated the same T:N, then the lowest dose, namely 0.5 mg/kg, was going to be selected for Phase B.

Based on Phase A data, Lumicell developed a dose-response model (see Appendix C) to predict the effects of dose on T:N and signal variations. Based on these results, the dose of 1.0 mg/kg was selected for Phase B.

Subjects in Phase B will be injected with a dose of 1.0 mg/kg. Phase B will enroll **10 evaluable subjects** with positive margins based on histological margin assessment from the initial shaved margin (with a **maximum enrollment of up to 50 subjects to achieve 10 subjects with positive margins**).

The total enrollment of the Feasibility study is 25 to 65 subjects (15 subjects in Phase A and up to 50 subjects in Phase B). The endpoints for the Phase A and Phase B of the Feasibility study are outlined below.

Endpoints:

Phase A:

- Select the dose (either 0.5 mg/kg or 1.0 mg/kg) for Phase B and pivotal studies that generates the largest average tumor-to-normal tissue signal ratio.
- Define the parameters for the detection algorithm to be used in the Phase B portion of the study.
- Collect safety data

Phase B:

- Evaluate and, if necessary, adjust the detection algorithm parameters
- Adjust, if necessary, the protocol for the pivotal trial.
- Collect safety data

General Procedure: (Same for both Phase A and B)

The first group of 5 subjects in Phase A will not be injected with LUM015 but will undergo the procedure outlined below as if they were injected with LUM015. Because of their exposure to the LUM 2.6 Imaging Device, these initial 5 subjects will be followed and monitored for safety in the same manner as all the patients injected with LUM015. The second group of 5 subjects (and the third group of 5 subjects, if necessary) in Phase A and all subjects in Phase B will be injected with LUM015 between 2 to 6 hours prior to surgery at a dose of 0.5 mg/kg, or 1.0 mg/kg. The selection of these doses and the timing of injection prior to surgery are based on the results of an IND phase I study in sarcoma and breast cancer subjects undergoing tumor removal surgery. A summary of the IND phase 1 study can be found in Section 10 below. Subjects will be observed to collect safety data of the LUM Imaging System from the time of injection of LUM015 to the time they are discharged from the hospital. The subjects will have a final safety assessment at the first post-operative visit.

Patients will be injected with LUM015 2-6 hours prior to surgery. For patients with non-palpable tumors, an ultrasound or mammogram guided needle localization procedure will be performed after LUM015 injection. Patients having sentinel node biopsies will be injected with Technetium-99 per standard of care. The injection of Technetium-99 may occur before or after injection of LUM015. Note that methylene blue or any other blue dye used for sentinel node mapping will not be used in this study.

Prior to the surgery, the LUM 2.6 Imaging Device will be located in the surgery 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. These patients will receive standard of care treatment and will not be imaged with the LUM 2.6 Imaging Device. These patients will be followed and monitored for safety the same way as those patients that undergo imaging with the LUM 2.6 Device. These patients will be included in the safety cohort but will not be part of the imaging analysis (efficacy) cohorts.

During histology analysis by the pathologist, images from the histology slides will be recorded for concordance with the fluorescence imaging results from the LUM 2.6 Imaging Device.

Phase A Procedures:

1. LUM 2.6 Imaging Device is initialized.
2. For surgeries with sentinel lymph node resection:
    a. surgeon performs axillary incision
    b. surgeon records 3-4 images of normal appearing tissue within the axillary incision using the LUM 2.6 Imaging Device
    c. surgeon performs sentinel node resection procedures and sends node specimen for intraoperative analysis

   d. surgeon records whether any interference or adverse impact of LUM015 on the sentinel lymph node procedures.
3. Surgeon performs surgical incision in the breast.
4. Surgeon removes lumpectomy specimen (gross resection).
5. For non-palpable tumors, the lumpectomy specimen is imaged by x-ray at the OR suite. The surgeon will remove additional tissue (which is part considered part of the gross resection) if indicated by the x-ray imaging results.
6. Surgeon records 3-4 images of normal appearing tissue on the underside of the skin flaps using the LUM 2.6 Imaging Device. These images will be used for evaluation of baseline fluorescence of normal tissue.
7. Surgeon scans the tumor bed cavity (TB1, **Figure 2**) with the LUM 2.6 Imaging Device and research assistant records images from every surface. The surgeon is blinded to the images.
8. Surgeon removes one shaved margin specimen from each tumor bed cavity surface per standard of care at MGH. Final margin assessment is based on pathology analysis of this shaved margin specimen.
9. Surgeon scans the tumor bed cavity (TB2) with the LUM 2.6 Imaging Device and research assistant records images of every surface. The surgeon is blinded to the images.
10. Standard of care surgery continues.
11. Surgeon or research assistant records images from the 6 faces of the lumpectomy specimen ex vivo using the LUM 2.6 Imaging Device.
12. Surgeon or research assistant records images of both sides of the shaved margin specimens (TB and M surfaces) ex vivo using the LUM 2.6 Imaging Device and marks with purple ink areas highlighted as suspicious by the LUM Device.
13. Surgeon or pathologist inks and then transects the lumpectomy specimen and records an image from the cross section of the specimen.

The table below shows the data to be collected. Note that TB1 will be imaged twice: once in vivo in the lumpectomy cavity and once ex vivo in the shaved margin to account for potential spatial differences between the in vivo and ex vivo tissue images.

Table 1: Imaging and pathology data to be collected in the Feasibility Phase A study.

Location	Lumpectomy specimen (ex vivo)		Lumpectomy cavity TB1 (in vivo)	Shaved cavity margin		Lumpectomy cavity TB2 (in vivo)
	M1	Transection		TB1 (ex vivo)	M2 (ex vivo)	
Anterior	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only
Posterior	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only

Superior	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only
Inferior	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only
Medial	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only
Lateral	Imaging and pathology (when available)	Imaging and pathology (when available)	Imaging only	Imaging and pathology	Imaging and pathology	Imaging only

Final margin assessment will be done by radial sectioning of the cavity shavings to examine the M2 surface per standard pathology protocols.

Imaging data with their respective histologic assessment for the presence of abnormal tissue were used to generate receiver operator characteristics (ROC) curves based on Lumicell's abnormal tissue detection thresholds described below.

From the Phase A imaging data, Lumicell used the metric of TB1000, which is defined as the minimum signal from the brightest contiguous cluster of 1000 μm diameter in the field of view. We selected the TB1000 metric as it represents true spatial characteristics of foci of cancer and is likely to give the best results going forward. The data from Phase A showed relatively small patient-to-patient variations in signal, thus a fixed, absolute threshold can be used to discriminate between tumor and normal tissue images. The fixed threshold is represented as:

Tumor present if TB1000 signal in a given tissue image > ***Y***

Although our Phase A data shows that a fixed threshold works well with the small population tested, it is possible that with a larger population we may encounter larger patient-to-patient variations. Thus, Lumicell decided to plan for the possibility of needing a threshold that varies proportional to normal tissue signal. This patient-specific variable threshold is represented as:

Tumor present if TB1000 signal in a given tissue image > ***a*****subject's normal tissue signal baseline*

The normal tissue signal baseline is determined by calculating the 25^{th} percentile for each of the in vivo images recorded before the surgeon removed the standard of care cavity shave and selecting the lowest value among them.

Lumicell's software compares the fixed threshold with the patient-specific variable threshold for each patient and will select the lower of the two to determine whether tumor is present in the tissue until one threshold approach proves to be superior.

Phase B Procedures:

1. LUM 2.6 Imaging Device is initialized.
2. For surgeries with sentinel lymph node resection:
    a. surgeon performs axillary incision
    b. surgeon records 3-4 images of normal appearing tissue within the axillary incision using the LUM 2.6 Imaging Device
    c. surgeon performs sentinel node resection procedures and sends node specimen for intraoperative analysis
    d. surgeon records whether any interference or adverse impact of LUM015 on the sentinel lymph node procedures.
3. Surgeon performs surgical incision in the breast.
4. Surgeon removes lumpectomy specimen (gross resection).
5. For non-palpable tumors, the lumpectomy specimen is imaged by x-ray at the OR suite. The surgeon will remove additional tissue (which is part considered part of the gross resection) if indicated by the x-ray imaging results.
6. Surgeon records 3-4 images of normal appearing tissue on the underside of the skin flaps using the LUM 2.6 Imaging Device. The software will use these images to set the abnormal tissue detection threshold using the algorithm confirmed during Phase A.
7. Surgeon scans the tumor bed cavity (TB1) with the LUM 2.6 Imaging Device and research assistant records images from every surface and takes note of the quadrants identified as containing fluorescence above the abnormal tissue threshold (these regions will be displayed in red in the screen). The surgeon is blinded to the images.
8. Surgeon removes one shaved margin specimen from each tumor bed cavity surface per standard of care at MGH. This shaved margin specimen will be analyzed by the pathologist to evaluate whether the subject had a positive margin after standard of care treatment.
9. Research assistant informs the surgeon of the imaging results from step 4.
10. Surgeon scans the tumor bed cavity (TB2) with the LUM 2.6 Imaging Device while surgeon and research assistant observe the real-time imaging results. Research assistant will record an image from each surface.
11. Surgeon removes an additional shaved margin specimen from any surface showing residual fluorescence (highlighted in red in the screen). Surgeon will repeat the imaging procedure until no residual fluorescence is observed but will not remove more than a total of 2 cm of additional margin tissue in depth. Final margin assessment for each lumpectomy surface is based on pathology analysis of the final shaved margin specimen.
12. Surgeon or research assistant records images from the 6 faces of the lumpectomy specimen ex vivo using the LUM 2.6 Imaging Device and applies purple ink to areas showing fluorescence.

13. Surgeon or research assistant records images of both sides of the shaved margin specimens (TB and M surfaces) ex vivo using the LUM 2.6 Imaging Device and applies purple ink to areas of shaved margin specimens showing fluorescence.
14. Surgeon or pathologist inks and then transects lumpectomy specimen and records an image from the cross section of the specimen.
15. Standard pathology inks will be applied by the pathologists per standard of care to gross specimen and shave margin specimen surfaces over the ink marking fluorescence, allowing complete pathological correlation of fluorescence and standard pathology findings.

Imaging data from gross resection dissection, M1, TB1 and M2 with their respective histologic assessment (when available) for the presence of abnormal tissue will be used to preliminarily evaluate the feasibility of the detection algorithm in identifying residual cancer. In addition, data analysis will compare positive margin rates based on the first standard of care shaved cavity margin (M1) and the positive margin rate based on the final, image guided shaved margin. This data will support the endpoints for the subsequent pivotal study.

During Phase B, after every 10 subjects complete the study, the safety data will be reviewed before recruiting the next 10 subjects. After every 10 subjects, we will also evaluate the device functionality based on the initialization procedure results and any changes to correct the device settings/components will be made.

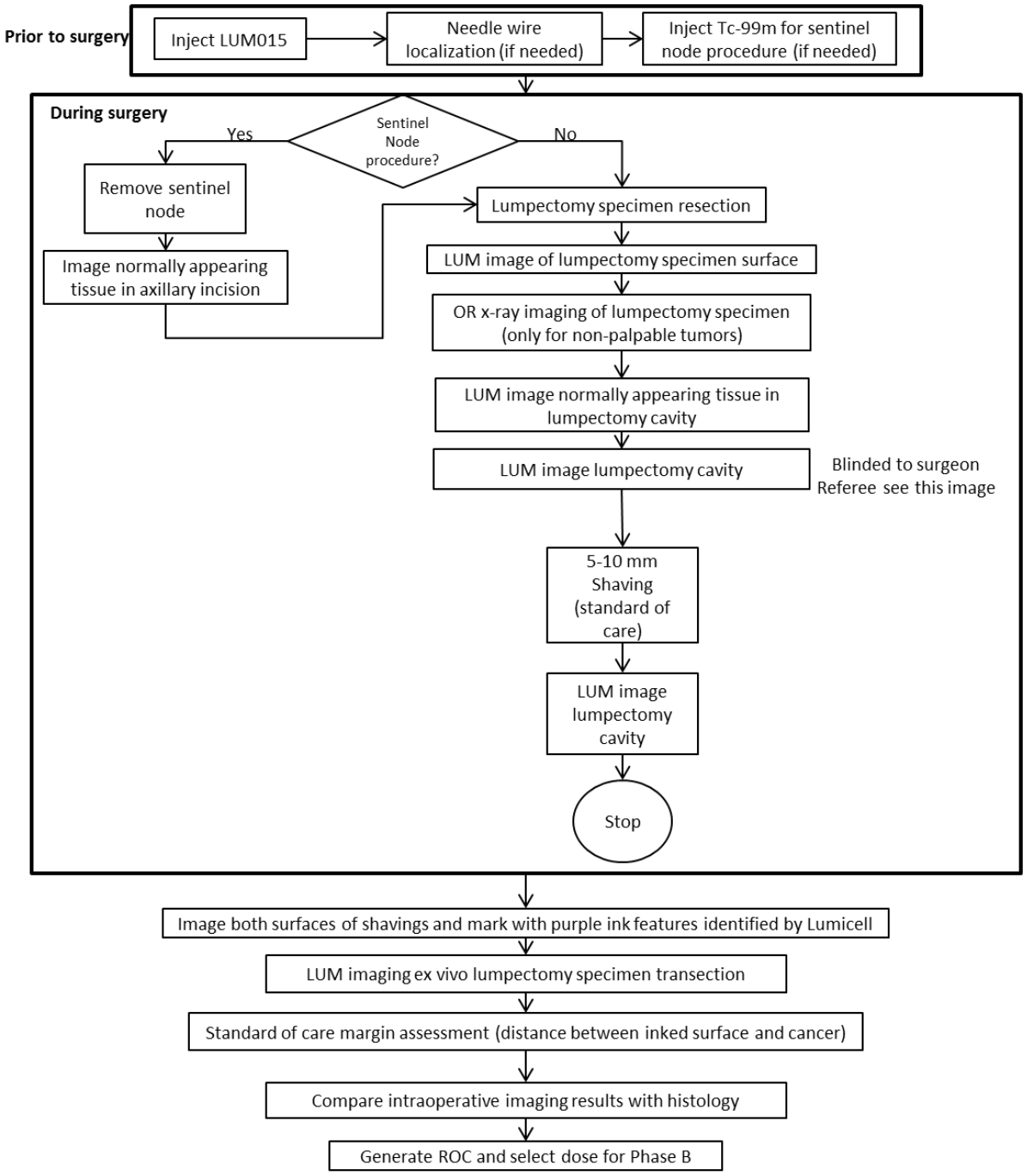
In some surgeries when the tumor is near the skin, a thin layer of tissue may be left just under the skin. Preliminary data shows that high fluorescence from the skin may create artifacts when imaging the thin layer of tissue in the lumpectomy cavity. In order to analyze possible artifacts caused by skin's fluorescence, we will perform imaging of the external breast tissue (skin) with the LUM Imaging Device pre and post injection of the LUM015 dye. Our intent is for a physician or research assistant to obtain a baseline signal background measurement by placing the LUM Imaging Device on ipsilateral and contralateral breast skin. Images will be taken prior to injection to within 30 minutes post injection. This data may provide information for developing the patient-specific tumor detection algorithm. If the physician or research assistant deems that this procedure will add additional stress, the external breast imaging will not occur.

Pathology analysis:

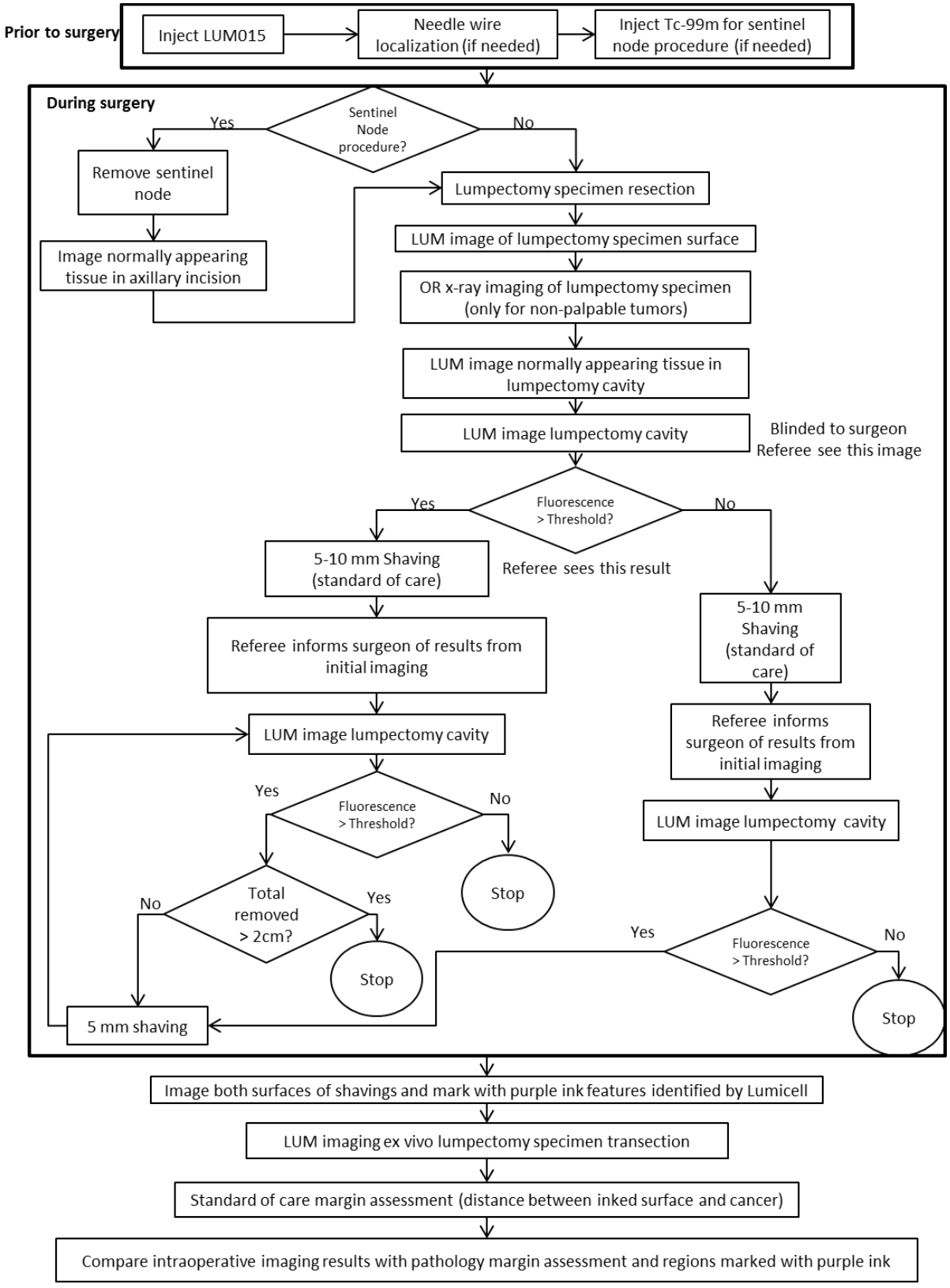
The purple ink applied during the ex vivo imaging procedures with the LUM 2.6 Imaging Device will not interfere with standard pathology procedures (see Appendix B for a detailed pathology protocol). The purple ink will be applied to both surfaces of the shaved margin, except on the final shaved margin in which the purple ink will be applied only to the TB surface. All the resected specimens will be inked and processed per standard of care pathology procedures. After all the standard of care pathology procedures are completed, the study pathologist will review histopathology of areas marked with purple ink that designate areas of fluorescence identified by the LUM 2.6 Imaging Device and correlate fluorescence with histopathology findings. See Appendix B for details of the pathology procedures.

1.2 Schema

Feasibility study Phase A (n=15 subjects)



Feasibility study Phase B (n=10-50 subjects)



1.3 Objectives

Primary objectives of the Feasibility Study:
(1) To determine the dose to be used in the pivotal trial; (2) to evaluate the detection algorithm for identifying residual cancer in the tumor bed; and (3) to gather additional safety data of the LUM Imaging System in breast cancer subjects.

2. BACKGROUND

2.1 LUM Imaging System

The Lumicell imaging system is a combination product consisting of an imaging device and an imaging agent (LUM015).

2.1.1 LUM Imaging Device

The LUM Imaging Device consists of a computer control unit, monitor and light source mounted in a cart, and a hand-held imaging head (LUM 2.6 imaging head). 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 3**) was designed as a lightweight hand-held tool with a small profile to allow easy maneuverability and limited intrusiveness into the operating room. The imaging head has 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 used to cover the optical head, which comes into contact with both the surgeon and the patient's exposed tumor bed. The sterile barrier assembly consists of a pre-assembled 1) sterile, disposable optical window made of plastic with a glass insert, which maintains the imaged tissue at the focal plane of the imaging system and 2) sterile, disposable plastic drape (510(k) clearance number K964142). The disposable optical window and drape are installed on the LUM Optical Head in the OR following aseptic procedures just prior to the imaging procedures.

Lumicell's intraoperative imaging approach consists of several steps: (1) acquire wide field of view image with microscopic resolution; (2) identify abnormal tissue using a computational algorithm; and (3) provide feedback to the surgeon by displaying the location of the abnormal tissue in an image (monitor) or indicating that the image of the zone does not have a positive indication of cancer tissue. Most importantly, all these steps are accomplished in real-time.

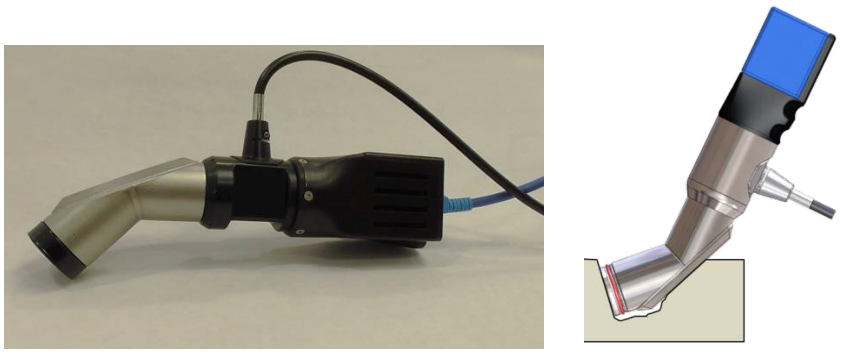


Figure 3: (Left) Photo of the LUM 2.6 imaging head. (Right) Rendering of the LUM hand-held imaging head scanning a lumpectomy cavity. The sterile transducer cover over the device is not shown.

The LUM 2.6 imaging head is powered by Lumicell's proprietary detection software which processes the fluorescence images, determines whether any tumor margin cells are in the field of view and highlights them. The software performs corrections for camera dark counts and uneven illumination to improve performance across the entire field of view. The software uses images of normal tissue to set the threshold to identify normal from abnormal tissue. When the surgeon scans the tumor bed, the detection software compares the intensity of an image against the threshold and identifies whether a region of the image contains abnormal tissue. Regions with signal above the threshold are displayed as highlighted regions on the monitor. Figure 3 shows an image of actual residual cancer (**Figure 4a** and **b**) along with what the surgeon sees (**Figure 4**c) in real-time with Lumicell's algorithm indicating the regions with residual cancer to be removed. The algorithm was developed based upon hundreds of tissue images from mice, dogs and humans compared against pathology of the imaged tissue. Based on a typical image size of ~6 Mbytes and a transfer rate of 10 Hz, the nominal requirements for the computer are: 2.0 GHz processor, 512 Mbytes of RAM, and operates in a Windows environment. The image acquisition and analysis software is written in C++.

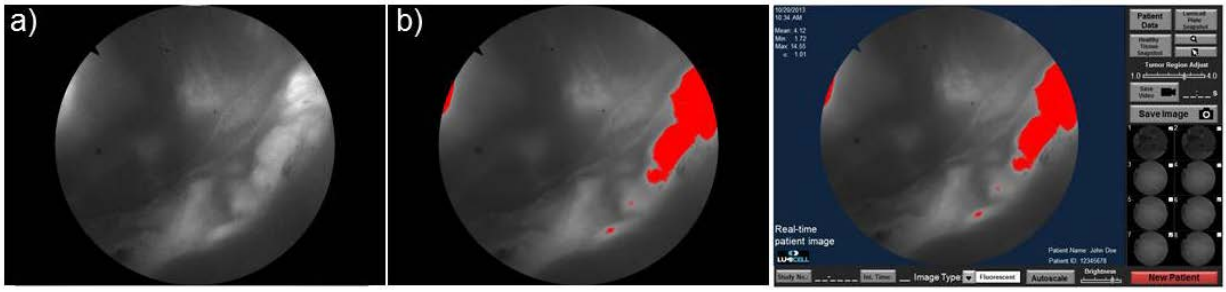


Figure 4: (a) A fluorescence image is shown that was taken from a mouse-sarcoma tumor bed after surgery in a mouse following IV injection of LUM015. (b) The same image was analyzed by Lumicell's detection system and regions containing residual cancer are highlighted in red. (c) This panel shows the user interface that the surgeon will see.

2.1.2 LUM015

Lumicell engineered LUM015, a fluorescence-based imaging agent that accumulates within the tumor and the tumor invasive front and is activated by cathepsin enzymes at these locations to label regions that should be removed during surgery. In its native state, the fluorescence of LUM015 is suppressed by an internal quencher molecule (QSY21); however, once cathepsin proteases cleave LUM015 at its amino acid backbone, the quencher is released and the fluorescent dye Cy5 in LUM015 emits detectable fluorescence (**Figure 5**). The absorption and emission wavelength maxima for the fluorescent molecule used, Cy5, are 649 nm and 670 nm respectively. LUM015 employs a fluorescent molecule with excitation and emission in the far red spectrum (< 700 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 [2].

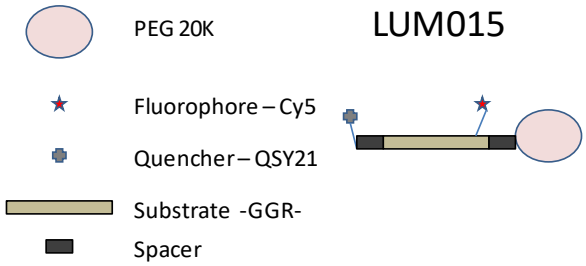


Figure 5: Schematic representation of LUM015.

Cathepsins are a family of enzymes that are involved with 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 invasive front [3-7]. Platt et. al. shows 60-fold higher cathepsin activity in breast cancer tissue than in healthy breast tissue [8]. 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) [4, 8-11]. Also, cathepsin B is typically over-expressed in inflammatory breast cancer, the most lethal form of primary breast cancer with a 3-year survival rate of 40% [12, 13]. 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 detected with the LUM Imaging System to identify cancer and cancer-related cells from adjacent normal tissue.

Lumicell's imaging agent application is unique as its properties allow the detection of cancer cells at or just below the surface of the exposed tumor bed to be consistent with the margin assessment performed by pathology. For this protocol, positive margins are declared when cancer cells are present at the inked surface.

The safety of LUM015 has been investigated under IND 111,670, in a study titled: "A Phase 1 Study of the Safety and Activation of a Cathepsin-activatable Fluorescent

Cancer Specific Probe LUM015." The IND Sponsor is David G. Kirsch, MD, PhD, Associate Professor of Radiation Oncology at Duke University School of Medicine where the study was being conducted. The device component of the system was not used on subjects in the Phase I study, but was used to image excised tissue in the pathology suite. No clinical treatment decisions were made on the basis of this imaging during the Phase 1 study.

The Phase I study has been completed with fifteen subjects participating in the trial (see Table 2 below for a summary of the study cohorts) under the IND 111670. No adverse pharmacological activity (APA) or adverse events were observed in any of the subjects. Tissues from these subjects were imaged ex vivo using Lumicell's current device and we determined an average tumor signal to background (normal tissue) signal ratio (TBR) of 5:1 (Table 3).

Table 2: Summary of the Phase I study cohorts.

Cohort	Number of patients	Dose (mg/kg)	Imaging time point (hr)
1	3	0.5	~29
2a	3	1.0	~29
2b	3 (1 breast cancer subject)	1.0	~6
3	3 (1 breast cancer subject)	1.5	~6
4	3 (1 breast cancer subject)	0.5	~6

2.1.3 Use of the LUM Imaging System

LUM015 is reconstituted in 0.45% saline at the institution's pharmacy prior to the injection and is stable for 4 hours when stored at room temperature or 24 hours when stored refrigerated between 2°C and 8°C. Following the instructions for use, a calculated amount of solution volume of LUM015 is obtained based on the dose and the patient's most recently obtained body weight and placed in a sterile syringe. LUM015 is administered into the patient via slow intra-venous push 2 to 6 hours prior to the surgery.

Prior to starting the surgery, the LUM Imaging Device will be located at the operating room and powering up routines and calibrations will be performed by OR personnel according to the instructions for use (IFU). A sterile imaging tip and drape will be installed over the device to provide a barrier between the imaging head and the sterile field. During surgery, once the surgeon removes the bulk tumor mass, the surgeon will place the LUM Imaging Head in contact with normal appearing tissue within the surgical incision to obtain a fluorescence baseline. Then, the surgeon will scan the tumor bed cavity. Real-time images are displayed in gray-scale and regions with residual fluorescence identified as abnormal tissue by Lumicell's detection algorithm will be highlighted in red in the screen (**Figure 4**). The surgeon will then take shavings from the tumor bed cavity regions indicated to contain abnormal tissue.

Final margin assessment by a pathologist is conducted based on the last shavings removed.

To date, the intraoperative imaging system has been tested in mice and dogs and post-surgery in humans and achieved superb sensitivity and specificity across these species and several cancer types as shown in Table 3. We define sensitivity and specificity when imaging is compared against pathology of the imaged tissue.

Table 3: Lumicell's technology has high sensitivity and specificity across cancers and species.

Species and cancer type	Endpoint	Sensitivity and Specificity	Tumor-to-normal tissue signal ratio
Humans (60 tissue samples)	Pathology of resected tissue	100%, 71%	5:1
Dogs with lung, mammary gland, sarcoma, mast cell tumors (96 tissue samples for 21 subjects)	Pathology of resected tissue and negative margins	93%, 95%	7:1
Mice with sarcoma (n=105)	Pathology of resected tissue	90%, 80%	8:1
Mice with sarcoma (n=34)	Local recurrence	80%, 80%	8:1
Mice with breast cancer (n=44)	Pathology of resected tissue	100%, 100%	8:1

2.2 Study Disease

We propose to conduct a clinical trial to test the performance of the LUM Imaging System during breast tumor resection to ensure that negative margins are obtained during the initial surgery thus eliminating the need for a second resection. 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. If tumor cells are found near or at the edge of the resected tissue, the patient is considered to have close or positive margins. Positive lumpectomy margins are the most important risk factor for local recurrence [14-16] and dictate that a second surgical procedure is required. Rates of close or positive margins have been reported in the range between 17 to 59% [17-21]. In two separate retrospective reviews of lumpectomies performed at the Massachusetts General Hospital between 1997 and 2007, we found rates for close or positive margins of 23% [22] and 36% [23]. Our review also found that 91% of patients with positive margins had re-excision surgeries followed by adjuvant radiation therapy. Furthermore, approximately 32% of patients having either one or two re-excisions still had close or positive margins. We estimate that in the US more than 150,000 women undergo lumpectomy for breast cancer each year and approximately a third of them require additional surgery for positive margins. Thus, a diagnosis of

positive margins indicates that additional treatments are needed which places a heavy burden on patients and may be a large added cost to the healthcare system.

2.3 Rationale

Detection of negative margins during tumor excision is critical to assure all the cancer has been removed from the tumor bed. Current standard of care dictates that tissue removed during surgery is analyzed post-operative by a pathologist as described above. This procedure is time consuming, often requiring 7-10 days of pathological 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. Thus, a safe method to directly assess the entire tumor bed and identify residual microscopic disease for resection intraoperatively would be highly beneficial for patients.

Lumicell has developed the LUM Imaging System, a fluorescence-based imaging agent and a hand-held, wide-field detector that can image the lumpectomy cavity in seconds to identify microscopic residual disease. The technology has demonstrated single cell detection and is insensitive to the natural motions of the surgeon or the patient. Therefore, this technology may enable surgeons to achieve negative margins during the initial surgery and eliminate the need for re-excision surgeries. The detection system images the tumor bed, overcoming tissue handling and sampling limitations of standard pathology analysis of resected tissue. The LUM imaging head is compact, provides real-time feedback to the surgeon about the status of the tumor bed, and does not require patient immobilization; hence, the technology does not have a significant impact on the surgical workflow. With this technology, breast cancer surgeons can make decisions that have the potential to prevent repeat surgeries, minimize patient discomfort, and reduce surgical risks.

Lumicell has demonstrated safety and efficacy in over 100 mice and 21 dogs (currently running a clinical trial in canine subjects at Tufts University Cummings School of Veterinary Medicine with Dr. John Berg and the Veterinary Specialty Hospital of the Carolinas with Dr. William Eward).

The current protocol describes a clinical trial to evaluate the efficacy of the LUM Imaging System in achieving negative margins in breast cancer subjects. In the initial feasibility study, we will finalize the algorithm used in the LUM Imaging System to detect residual cancer when compared against histology. In subjects from the Pivotal Study, we will prospectively evaluate the efficacy of the technology to detect residual cancer and reduce the rates of post-operative positive margins.

2.4 Correlative Studies Background

Not applicable.

3. SUBJECT SELECTION

3.1 Eligibility Criteria

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

3.1.1 Subjects must have histologically or cytologically confirmed primary breast cancer. The methods for obtaining the histological samples can include diagnostic biopsies, core needle biopsies or fine needle biopsies.
3.1.2 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.
3.1.3 Subjects must be scheduled for a lumpectomy of a breast malignancy.
3.1.4 Subjects must be able and willing to follow study procedures and instructions.
3.1.5 Subjects must have received and signed an informed consent form.
3.1.6 Subjects must be otherwise healthy except for the diagnosis of cancer, as per the exclusion criteria listed below.

3.1.7 Subjects must have normal organ and marrow function as defined below:

- Leukocytes $\geq$ 3,000/mcL
- Platelets $\geq$ 100,000/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.

3.1.8 The effects of LUM015 on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) starting the day entering the study, and for 60 days after injection of the imaging agent. 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 pregnancy during treatment, so this requirement does not differ from standard of care.
3.1.9 Subjects with ECOG performance status of 0 or 1.

3.2 Exclusion Criteria

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

3.2.1 Subjects with a known current condition of substance addiction.
3.2.2 Subjects who have taken an investigational drug within 30 days of enrollment.
3.2.3 Subjects with prolonged QT interval.
3.2.4 Subjects who will have administration of methylene blue or any blue dye used for sentinel node mapping procedures prior to imaging the lumpectomy cavity with the LUM 2.6 Imaging Device.
3.2.5 Subjects who have not recovered from adverse events due to pharmaceutical or diagnostic agents.
3.2.6 Subjects with uncontrolled hypertension defined as persistent systolic blood pressure > 150 mm Hg, or diastolic blood pressure > 95 mm Hg; those subjects with known HTN should be stable within these ranges while under pharmaceutical therapy.
3.2.7 Subjects with insulin dependent diabetes mellitus.
3.2.8 History of anaphylactic reaction attributed to any contrast agent or drugs containing polyethylene glycol (PEG).
3.2.9 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.
3.2.10 Pregnant women are excluded from this study 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. These potential risks may also apply to other agents used in this study.
3.2.11 Subjects who are sexually active and not willing/able to use medically acceptable forms of contraception upon entering the study; this exclusion is necessary because the teratogenic properties of the study imaging agent are unknown.
3.2.12 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with LUM015.
3.2.13 Any subject for whom the investigator feels participation is not in the best interest of the subject.
3.2.14 Subjects undergoing a second surgery because they had positive margins in a previous surgery.
3.2.15 Subjects with prior ipsilateral breast surgeries, mastectomies, breast reconstructions or implants. (Note: subjects who have had prior breast biopsies are not excluded; see inclusion criterion 3.1.1.)
3.2.16 Subjects with prior ipsilateral reduction mammoplasties or breast reductions performed less than 2 years prior to enrollment to this study.

3.2.17 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.

3.3 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 likely to be eligible for this study.

4. SUBJECT ENROLLMENT

4.1 General Guidelines for Screening and Enrollment

The Institution will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the QACT protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

The study team will document subject eligibility on the Screening and Enrollment form and notify Lumicell of a potential candidate for the study after the subject has been consented.

The subject is not considered enrolled into the study until written Informed Consent is obtained and the investigational product has been administered (Study Day One).

Lumicell will perform an analysis of the data collected in Arms 1 and 2 to determine if the acceptance criterion is met (normal tissue signal is separated by 4 standard deviations from the mean autofluorescence in normal tissue) and an additional 5 subjects (Feasibility Study Phase A, Arm 3) will be enrolled in the study if the acceptance criterion is not met. Lumicell will notify the clinical site when enrollment can restart.

4.2 Enrollment Process

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin

treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

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. Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the subject's medical/research record. **To be eligible for registration to the study, the subject must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

6. Notify Lumicell clinical team of eligible subject status and date of proposed surgery.

5. INTERVENTIONAL PLAN

The investigational imaging agent LUM015 will be administered in the hospital setting prior to surgery as described below. No investigational or commercial agents or therapies other than those described below may be administered with the intent to image the subject's malignancy.

LUM015 will be administered as a single dose via peripheral intravenous (IV) injection. Prior to injection, the line must be flushed with 10-20 mL of saline. The injection of LUM015 is immediately followed by a saline flush of 10-20 mL. The dosage of LUM015 is 0.5 mg/kg or

1.0 mg/kg. LUM015 will be diluted in 0.45% saline without glucose. LUM015 will be administered between 2 to 6 hours prior to the scheduled surgery.

5.1 Pre-treatment Criteria

No pre-treatment is required.

5.2 Agent Administration

5.2.1 LUM015

- Administration – The intravenous (IV) line through which LUM015 will be injected must be flushed with 10-20 mL of saline just prior to injection of LUM015. LUM015 is administered as a slow push rate over 3 minutes in a single dose of 0.5 mg/kg or 1.0 mg/kg via peripheral IV injection in 0.45% saline without glucose between 2 to 6 hours prior to surgery. The injection is immediately followed by a saline flush of 10-20 mL.
- Dosing – LUM015 is provided in amber vials in lyophilized form. Nominally each vial contains 10 mg of LUM015, 10 mg of mannitol, 0.83 mg of sodium phosphate monobasic and 0.43 mg of sodium phosphate dibasic. Preparation of the injection dose is explained in detail in Appendix A. Upon reconstitution with 1.0 mL of 0.45% saline without glucose, the pH of the solution is 6.5.
- 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. All study safety assessments will continue until the first post-operative visit (approximately 2-3 weeks after surgery).

5.2.2 Other Modality(ies) or Procedures

5.2.2.1 Subjects in Feasibility Phase A:

The first cohort of patients in Phase A were not injected with LUM015 and underwent the procedure outlined below as if they were injected with LUM015. The second and third cohorts in Phase A were injected with LUM015 between 2 to 6 hours prior to surgery at a dose of 0.5 mg/kg (n = 5 subjects), or 1.0 mg/kg (n = 5 subjects). The selection of these doses was based on the results of a phase I study in sarcoma and breast cancer subjects undergoing tumor removal surgery. Subjects were observed to collect safety data of the LUM Imaging System from the time of injection of LUM015 to the time they were discharged from the hospital. The subjects had a final safety assessment at the first post-operative visit. After standard of care gross tumor resection, the surgeon recorded 3-4 images from visually normal appearing tissue with the LUM imaging head. The surgeon recorded these images with the LUM imaging head from the anterior, posterior, medial, lateral, inferior and superior quadrants of the lumpectomy

cavity. All images were labeled according to the location from where they were taken in the lumpectomy cavity. No clinical decisions were made based on the imaging results and the surgeon was blinded to the all the imaging results to avoid bias towards excision of more than standard amounts of shaved cavity margin tissue. Following standard of care practices, the surgeon then removed an additional 5-10 mm thick strip of tissue (shaved cavity margins) from the walls of the lumpectomy cavity for histological margin assessment. After the standard of care strip of tissue was removed, the surgeon recorded images with the LUM imaging head from the newly exposed anterior, posterior, medial, lateral, inferior and superior quadrants of the lumpectomy cavity. All images were labeled according to the location from where they were taken in the lumpectomy cavity. An image was recorded from the inked surface of the additional strip of tissue removed. Images were also recorded from the gross resection. Then a pathologist assistant dissected a portion of the gross resection and recorded an image from the inside of the resection.

5.2.2.2 Subjects in Feasibility Phase B

Subjects undergoing breast cancer surgery will be injected with LUM015 between 2 to 6 hours prior to surgery at a dose of 1.0 mg/kg as determined from the results of the feasibility study Phase A. Subjects will be observed to assess the safety of LUM015 from the time of injection to the time they are discharged from the hospital. After standard of care gross tumor resection, the surgeon will record images with the LUM imaging head from the anterior, posterior, medial, lateral, inferior and superior surfaces of the lumpectomy cavity. These images will be used to determine the threshold based on the normal tissue multiplier. Then the LUM Imaging System software compares this threshold against the fixed threshold and selects the lowest value as the threshold for that patient. All images will be labeled according to the location from where they were taken in the lumpectomy cavity. The surgeon will be blinded to this imaging result to avoid bias towards excision of more than standard amounts of shaved cavity margin tissue, but a technician at the instrument console will record whether the imaged tissue was classified as normal or abnormal by the LUM Imaging System. After the standard of care removal of the 5-10 mm thick strip of additional tissue, the technician will reveal the results from the first imaging to the surgeon. The surgeon will then record images from the new lumpectomy cavity's walls and will resect another 5-10 mm strip of tissue from any surface identified as having abnormal tissue. The surgeon will limit the extent of additional tissue resected to 20 mm in thickness or less to minimize the cosmetic impact of the additional resection.

In some surgeries when the tumor is near the skin, a thin layer of tissue may be left just under the skin. Preliminary data shows that high fluorescence from the skin may create artifacts when imaging the thin layer of tissue in

the lumpectomy cavity. In order to analyze possible artifacts caused by skin's fluorescence, we will perform imaging of the external breast tissue (skin) with the LUM Imaging Device pre and post injection of the LUM015 dye. Our intent is for a physician or research assistant to obtain a baseline signal background measurement by placing the LUM Imaging Device on ipsilateral and contralateral breast skin. Images will be taken prior to injection to within 30 minutes post injection. This data may provide information for developing the patient-specific tumor detection algorithm. If the physician or research assistant deems that this procedure will add additional stress, the external breast imaging will not occur.

5.3 Definition of Dose-Limiting Toxicity

Not applicable.

5.4 General Concomitant Medication and Supportive Care Guidelines

A complete listing of all medications (including non-prescription, vitamins, herbal products and essential oils) taken within 24 hours of the first dose will be obtained at screening and updated as needed at enrollment. Concomitant medications will be recorded in the medical record and case report form.

5.5 Duration of Therapy

The imaging agent LUM015 will be injected as a single dose between 2 to 6 hours prior to surgery. 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.

5.6 Duration of Follow Up

All study intervention will be completed at the end of the surgical procedure. The initial five subjects who were not injected with LUM015, as well as all the subjects injected with LUM015 will continue to be monitored until hospital discharge. All subjects will continue their enrollment and be followed in the study until their medical team determines that no further surgical intervention is required. When attending their first post-operative visit a blood draw will be collected for a CBC (red blood cells, white blood

cells and platelets) and serum chemistry (alkaline phosphatase, total bilirubin, BUN, calcium chloride, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium) 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.

5.7 Criteria for Removal from Study

Subjects will be removed from study when any of the criteria listed in Section 5.5 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 will be replaced.

Individual patients may be discontinued from the study by the Investigator or the Sponsor 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 be discontinued if they become pregnant or withdraw consent. Patients may be discontinued due to noncompliance with the protocol. 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 the Lumicell Medical Safety Monitor:
Shelley Hwang, MD, MPH
Duke University Medical Center
Phone: 919-684-6849
Fax: 919-684-6044

5.8 Criteria for Stopping the Study

The events listed below are the stopping rules criteria for the study. In the case of any of these events, no additional patients will be recruited, dosed or imaged until an explanation can be found.

- Clinically significant laboratory findings that are deemed serious or severe (this includes if a trend is noted in clinically significant laboratory findings, see section 10.3)
- Any unanticipated adverse device effect (as defined in section 11.1.4)

- Hypersensitivity or allergic reactions of Grade 2 or greater as defined in CTCEA 4.03
- Any serious adverse event (as defined in section 11.1.2) that is considered either possibly, 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 Investigator.

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 by the sponsor to resume the study, approval from the FDA and IRB must be obtained prior to resuming the study.

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.
- Sponsor decision to terminate development.

6. DRUG FORMULATION AND ADMINISTRATION

6.1 LUM015

6.1.1 **Description**

LUM015 has an abbreviated chemical formula of QSY21-Ahx-GGRK(Cy5)-AEEAc-C(mPEG20,000), where QSY21 is a fluorescence quencher from Life Technologies, Ahx is aminocaproic acid, G is the amino acid glycine, R is the amino acid arginine, K(Cy5) is the amino acid lysine conjugated with the fluorescent dye Cy5 (GE Healthcare), AEEAc is amino-ethoxy-ethoxy-acetyl, C is the amino acid cysteine and mPEG20,000 is a chain of methoxy-polyethylene glycol with an average molecular weight of 20,000 g/mol. The appearance of LUM015 is blue. The molecular weight of LUM015 is approximately 22,000 g/mol.

6.1.2 **Form**

LUM015 is supplied in 3-mL vials in powder form containing approximately 10 mg of LUM015, 10 mg of mannitol, 0.83 mg of sodium phosphate monobasic and 0.43 mg of sodium phosphate dibasic. LUM015 is provided by Lumicell, Inc.

6.1.3 **Storage and Stability**

LUM015 must be stored at -20 °C (freezer) in powder form in the dark. Under these conditions, LUM015 is expected to be stable for up to 2 years. After reconstitution, LUM015 should be administered to the patient within 4 hours when stored at room temperature or 24 hours when stored refrigerated between 2°C and 8°C. Temperature logs for the storage freezers and refrigerators at the site's pharmacy will be kept.

6.1.4 **Compatibility**

Prior to injection, LUM015 should be reconstituted with 1.0 mL of 0.45% saline without glucose.

6.1.5 **Handling**

LUM015 should only be handled by qualified personnel, familiar with procedures for reconstituting and injecting drugs or agents. Exposure to direct light for more than 5 minutes should be avoided.

6.1.6 **Availability**

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

6.1.7 **Preparation**

LUM015 is provided in amber vials in lyophilized form. Nominally each vial contains 10 mg of LUM015, 10 mg of mannitol, 0.83 mg of sodium phosphate monobasic and 0.43 mg of sodium phosphate dibasic. LUM015 will be reconstituted by adding 1.0 mL of 0.45% saline without glucose. Detailed preparation of the injection dose is explained in Appendix A. Upon reconstitution the pH of the solution is 6.5 and the osmolarity is 270 m-osm/L.

6.1.8 **Administration**

LUM015 is administered as a single dose of 0.5 mg/kg or 1.0 mg/kg via peripheral intravenous (IV) injection in 0.45% saline between 2 and 6 hours prior to surgery.

The IV line must be flushed with 10-20 mL of saline just prior to injection of LUM015 and the injection is immediately followed by a saline flush of 10-20 mL.

6.1.9 **Ordering**

LUM015 will be stocked by Lumicell. Initially, Lumicell will supply vials of LUM015 for 10 subjects at the clinical site. When the stock of LUM015 at the pharmacy reaches the level to cover 5 subjects, authorized pharmacy staff will notify the Principal Investigator and Lumicell and Lumicell shall supply 5 more vials of LUM015. The goal is to have a stock of LUM015 for no less than 5 and no more than 10 subjects at a given time.

Lumicell shall be notified via electronic email at jmferrer@lumicell.com or by telephone at 617-571-0592 for ordering LUM015

6.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of LUM015 and imaging device utilizing documentation forms provided by Lumicell. Inventory and disposition of LUM015 must be kept on NCI Drug Accountability Record Forms.

6.1.11 **Destruction and Return**

A drug inventory (using NCI Drug Accountability Record Form) will be maintained by the clinical site's pharmacy. The inventory will include details of the materials received and a clear record of when they were dispensed and for which subjects. This inventory record shall indicate the quantity and description of all investigational materials on hand at any time during the study.

Each clinical site investigator will also maintain a device accountability record

At the end of the study, unused supplies of LUM015 and the investigational device must be returned to Lumicell. Any LUM015 destroyed according to institutional policies will be documented in the Drug Accountability Record Form.

7. CORRELATIVE/SPECIAL STUDIES

Not applicable.

8. STUDY CALENDAR

Baseline evaluations are to be conducted within 4-weeks prior to subject enrollment. All baseline assessments must be performed prior to administration of any study agent. All study assessments should be administered within ± 3 days of the protocol-specified date, unless otherwise noted. The study calendar below applies to the initial five subjects that are not injected with LUM015 and all subjects injected with LUM015 including those that do not undergo imaging due to a failure in the LUM 2.6 Imaging Device initialization.

	Pre- Enrollment /Screening	Day 1 / Enrollment	2-14 days after surgery	Routine follow up visit
LUM015 administration		X		
Informed consent	X			
History	X			
Concurrent meds	X			
Physical exam (Ht, Wt, BSA, VS)	X			
Surgery/intraoperative imaging		X		
Margin assessment			X	
CBC	X			X
EKG	X			
Serum chemistry [a]	X			X
Adverse event/adverse device effect evaluation		X		X
Tumor measurements	X		X	
Radiologic evaluation	X			
B-HCG	X[b]			

a: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Serum pregnancy test (women of childbearing potential).

9. MEASUREMENT OF EFFECT

Not applicable.

10. SAFETY

A Phase 1 IND study in sarcoma and breast cancer patients has been 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) have been injected with LUM015 with no adverse pharmacological activity reported (Table 2). The only noticeable effect in the study subjects has been the green discoloration of urine, which resolved within 12-24 hours post injection for most patients.

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 was 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). The Phase I study report will be available by the end of December 2014.

Initial pharmacokinetic data from the first 11 study subjects indicates that LUM015 is cleared with a half-life of 3.6 ± 0.6 hours (average ± standard deviation) (**Figure 6**).

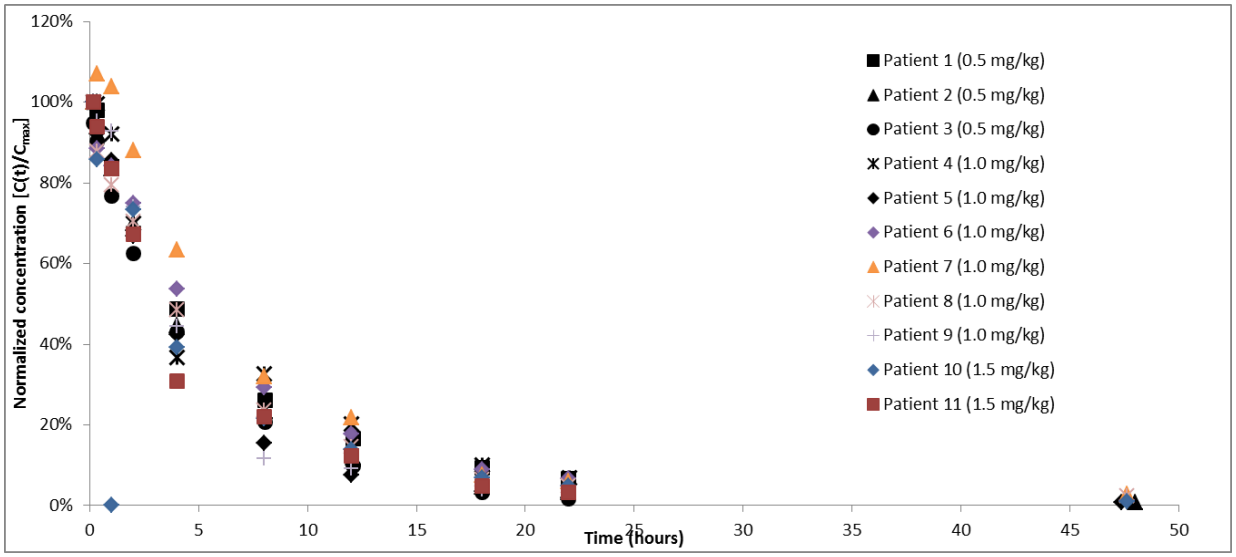


Figure 6: Normalized concentration of LUM015 in human plasma as a function of time after injection.

10.1 Expected Adverse Events (associated with LUM015)

No adverse events related to the administration of LUM015 were observed during the Phase 1 safety study. Prior to the Phase 1 study, LUM015 had not previously been used in humans. Our preclinical studies demonstrated that it was reasonably safe to proceed with a Phase 1 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. Notably, no hypersensitivity has been observed in the human patients that participated in the Phase 1 safety study.

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 2 (or greater) hypersensitivity or allergic reaction as defined in CTCEA 4.03, Lumicell should be contacted within 24 hours of the event:

To Lumicell:
Jorge Ferrer
Phone: 617-571-0592
Email: jmferrer@lumicell.com
fax: 781-672-2501

If this occurs, enrollment in the study should be held until after consultation with the FDA regarding the event is completed and a determination is made by the sponsor, principal investigator and FDA as to whether prophylactic treatment with diphenhydramine to subsequent subjects is warranted.

10.2 Monitoring of Laboratory Values

A CBC (red blood cells, white blood cells and platelets) and serum chemistry (alkaline phosphatase, total bilirubin, BUN, calcium chloride, glucose, LDH, phosphorous, potassium, total protein, SGOT [AST], SGPT [ALT], sodium) tests will be completed at the first post-operative visit during the routine follow up visit. We note that it is not standard of care to perform follow up blood tests unless abnormalities were identified prior to surgery. The investigator will review the laboratory reports for any abnormal post-operative laboratory valued and will determine if any results are clinically relevant.

Any abnormal values that are determined to be clinically significant will constitute adverse reactions and will be captured on the case report form. If a trend is noted of clinically significant laboratory findings (i.e. a specific event occurs in more than one patient), or if any serious adverse event that is possibly, probably or definitely related to LUM015 or the Imaging Device is reported, no additional patients will be recruited until an explanation can be found. The principal investigator will assess the likelihood that the adverse reaction or severe and/or serious adverse event resulted from LUM015. The FDA will be notified of any trends observed in clinically significant laboratory findings.

10.3 Expected Adverse Device Effects (associated with the LUM Imaging System)

To date, the LUM Imaging System has not been studied clinically. Thus, there are no known anticipated adverse device effects.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 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) 4.03.

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.

11.1.2 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

11.1.3 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.

11.1.4 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.

11.1.5 Expectedness

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

11.1.5.1 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.

Refer to Section 10.1 for a listing of expected adverse events associated with the study agent(s) and Section 10.4 for a listing of expected adverse device effects associated with the imaging device.

11.1.5.2 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.

11.1.6 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.

11.2 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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.

A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

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

11.3 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. It is the responsibility of the principal investigator to report serious adverse events to Lumicell and/or others as described below.

11.4 Reporting to Lumicell

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of LUM015 Imaging Agent must be reported to Lumicell using the provided SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

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

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the subject is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the subject is in long term follow up, report the death at the time of continuing review.

The principal investigator must report each serious adverse event to Lumicell within 24 hours of 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 within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

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

fax: 781-672-2501

11.4.2 Unanticipated Adverse Device Effects

All unanticipated adverse device effects (UADEs) that occur during the study are considered serious and must be reported to Lumicell using the provided SAE form. This includes events meeting the criteria outlined in Section 11.1.4.

The principal investigator must report each unanticipated adverse device effect to Lumicell within 24 hours of learning of the occurrence. In the event that the Investigator does not become aware of the unanticipated adverse device effect immediately (e.g., subject sought treatment elsewhere), the principal investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse device effect. Report unanticipated adverse device effects by telephone, email or facsimile to:

To Lumicell:
Jorge Ferrer
Phone: 617-571-0592
Email: jmferrer@lumicell.com
fax: 781-672-2501

11.4.3 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.

11.5 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 standard policy.

11.6 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.

11.7 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.

11.8 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, throughout the study, and within 30 days of the study intervention 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 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.

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 will complete the Protocol Deviation form provided in the Study Binder. Additionally, the reviewing IRB has to be contacted by the Investigator if a Protocol Deviation might either affect the participant's right, safety or well-being, or might significantly affect the completeness, accuracy and reliability of the data. The Protocol Deviation form provided by Lumicell will be used for this purpose.

Note: The Principal Investigator will summarize any protocol deviations noted during the course of the study in a final statement.

In the case that the deviation was identified by the Investigator, the Investigator will use the Lumicell deviation form to summarize and assess the impact of the deviation. Similarly, the Lumicell protocol deviation form will be utilized to communicate a deviation that is identified by Lumicell to the investigator. In both cases, the Investigator must provide Lumicell with a copy of the form submitted to their IRB or use the Lumicell form to document the deviation. The deviation will be categorized in the description section of the form as a Protocol Waiver or Protocol Deviation.

Examples of major Protocol Deviations include, but are not limited to the following:

- Inadequate or nonexistent informed consent
- Unreported Adverse Events/Adverse Device Effects
- Data Corruption or falsified data

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

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

12.1.2 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.

12.2 Safety Review

For this feasibility study, an independent safety reviewer will review and monitor adverse events data from this trial. The independent safety reviewer will be a clinical specialist 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.

After the completion of Phase A, the safety data from Phase A and from a repeat dose toxicity study in rabbits will be evaluated prior to starting recruitment of subjects for the Phase B. Phase B must not commence without approval from FDA. During Phase B, after every 10 subjects complete the study, the safety data and device performance data will be evaluated before recruiting the next 10 subjects.

12.3 Monitoring

Lumicell's monitoring process will be initiated with the initial visits to the site to assure that it meets the qualification requirements and has adequate experience to conduct the study protocol. When the site has met all qualification criteria, submitted all initial regulatory documentation, and received IRB approval for the study, Lumicell will conduct a study 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. 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; verify the investigator has approved of all study data submitted to Lumicell; 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, 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. Monitoring will begin at the time of subject registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 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.

13.2 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.

13.3 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
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- 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
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

    - Title 21 Part 812 – Investigational Device Exception Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- 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.

13.4 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.

Study Documentation Practices

The investigator must maintain adequate records to enable the conduct of the study to be fully documented.

a) 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.
b) The use of 'White-Out' or similar correction fluid is forbidden.
c) Handwritten dates are always to be recorded in the sequence of the month, day and year (mm/dd/yy or yyyy) unless otherwise specified.
d) Use of highlighters on records is acceptable so long as the highlighter color does not obscure the underlying text if the record is copied.
e) Accuracy is required. Always verify entries are correct and consistent with other information.
f) Signatures are to be authentic.
g) Recorded dates are to be the actual dates in which the activities were recorded. Back-dating is forbidden.
h) 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.
i) All forms must be filled out using non-erasable pen and must be legible. The use of blue or black ink is preferred.
j) 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.

13.5 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.

14. STATISTICAL CONSIDERATIONS

The primary objective of this clinical trial is to study the safety and efficacy of an intraoperative imaging system, the LUM Imaging System, to reduce the rate of breast cancer patients with post-operative positive margins. The safety of the imaging agent LUM015 was previously tested in a Phase I clinical trial conducted at Duke University Medical Center and the safety assessment will be continued in this study.

14.1 General Methods

For categorical variables, summary tabulations of the number and percentage of patients within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of patients, mean, median, standard deviation, minimum, and maximum values will be presented.

Each data value for all the included patients will be presented using patient data listings, sorted patient number, unless otherwise specified.

All data summary tabulations will be presented by study site and for the overall study population, as appropriate.

14.1.1 Disposition of Subjects

Demographic and baseline characteristics data will be summarized for all patients. The total number of patients screened, enrolled, completed and reason for termination will be summarized.

14.2 Study Design/Endpoints

Feasibility Phase A: The primary objectives for the feasibility trial are: (1) to find a dose of LUM015 that generates the highest average T:N, (2) to define the parameters for the detection algorithm and (3) to gather safety data.

Feasibility Phase B: The endpoints for the Feasibility Phase B are: (1) a preliminary evaluation of detection algorithm efficacy, and to make adjustments to the detection algorithm if needed, (2) make adjustments to the protocol for the pivotal trial, and (3) collect safety data.

Subjects undergoing lumpectomies will be injected with LUM015 at 1.0 mg/kg. The surgeon will perform standard of care surgery and then use the imaging system to take additional cavity shavings based on the detection algorithm output. We will recruit subjects until we obtain 10 evaluable subjects with positive margins based on histological assessment from the initial shaved margins. This group will allow for a maximum enrollment of up to 50 evaluable subjects based on an estimated positive margin rate of 20%. We believe that this number of subjects to test the detection algorithm will provide enough clinical data to adjust the parameters of the detection algorithm and move forward with the pivotal trial. We will generate an ROC to determine the parameters of the detection algorithm to be used in the pivotal trial.

14.3 Populations for Analysis

Safety evaluations will be based on all patients who enter the study including the initial five (5) patients who do not receive LUM015, and all the patients that receive LUM015. Efficacy evaluations will be based on the group of patients that complete the study.

14.4 Reporting and Exclusions

In some cases, there may be a margin shaving with a histological assessment but no fluorescence reading, or vice versa. Two analyses will be performed. In a "complete cases" analysis, only the shavings providing both measures will be analyzed. In a parallel "worst case" analysis, the fluorescence results will be assumed to be wrong. Both analyses will be reported, but the formal success criterion will be based on the complete cases analysis.

14.5 Safety Analysis

Adverse events and adverse device effects will be collected, analyzed and tabulated as needed. Serious adverse events (SAE) and unanticipated adverse device effects (UADEs) will be tabulated separately and analyzed with respect to their severity.

15. PUBLICATION PLAN

The principal investigator has the intent to publish the results of this study in a peer-reviewed journal. The results of this study will be made public within 24 months of the end of the data collection.

The results of this study may be submitted to regulatory authorities in support of product clearance. Any results of this study may be published or presented at scientific meetings. If this is anticipated, the Principal Investigator agrees to inform Lumicell and to submit all manuscripts or abstracts prior to submission. All publications and lectures with any reference to the subject of this agreement need the previous written consent of Lumicell.

Any formal publication of the study in which the input of Lumicell's personnel exceeds that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate Lumicell staff. Authorship will be determined by mutual agreement.

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17. APPENDICES

Appendix A: Protocol for preparation of LUM015 for injection

1 Purpose

The purpose of this protocol is to describe the dose preparation for the LUM015 Imaging Agent.

2 Materials

- LUM015 Formulated, stored frozen (-20°C)
- Sterile syringe
- 0.45% Saline

3 Methods

1) After reconstitution, LUM015 should be administered to the patient within 4 hours when stored at room temperature or 24 hours when stored refrigerated between 2°C and 8°C.
2) Obtain the most recent accurate weight of the subject in kg.
    - Note: If weight is measured in lbs, multiply value by 0.45359 to obtain kg. Example: 155lbs=(155*.45359)kg=70.306kg
3) Calculate total dose for subject according to protocol.
    - Example: If dose level is 1.0 mg/kg and weight is 70kg, then dose = 1.0*70=70 mg.
4) Obtain the required number of vials to administer the full dose. Each vial contains 10mg LUM015.
    - Example: If 70mg is the required dose, 70/10=7 vials.
    - Example 2: If the dose level is 1.0 mg/kg and the patient weight is 63 kg, the required number of vial for the dose of 63 mg is 63/10 = 6.3; thus 7 vials would be needed for this patient.
5) Allow bottles to acclimate to room temperature.
6) Using aseptic techniques:
    a) Remove protective cap from vial. See Figures 1 and 2 below.
    b) Draw 1mL of 0.45% saline into a sterile syringe.
    c) Puncture vial with syringe.
    d) Invert syringe and vial such that vial is located vertically up and syringe is closer to the floor.
    e) Draw 1mL of air from the vial into the syringe.
    f) Invert syringe and vial such that vial is located vertically down closer to the floor.
    g) Ensure air in syringe is located at the top, away from the floor.
    h) Push 1mL of fluid into the vial.
    i) Compound will dissolve instantaneously.
    j) Repeat steps a-i with remaining required vials.
    k) Remove fluid from each vial using one large syringe to combine all required fluid. Ensure that only the required fluid is obtained as the final concentration is

10mg/mL. Example: if 35mg is the required dose, remove only 35/10=3.5mL total fluid from all 4 vials.

7) Prep the injection site for the subject according to standard procedures.
8) Ensure the vial is homogeneous in blue color (see Figure 3 below) and that there are no air bubbles in the syringe.
9) The IV line must be flushed with 10-20 mL of saline just prior to injection of LUM015 and the injection is immediately followed by a saline flush of 10-20 mL.
10) Inject slow bolus into the subject (over three minutes).
11) Discard remaining solution and syringes.
12) Record subject weight, dose, compound lot number, and expiration date on accompanying subject case report form.

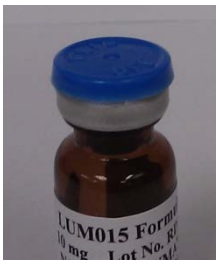


Figure 1: Vial with protective cap

Figure 2: Empty vial with protective cap removed

Figure 3: Dissolved compound

4 Caution

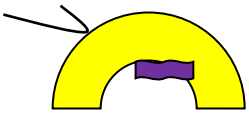
- **Ensure there are no air bubbles in the syringe prior to injection.**
- **Ensure there are no solid particles visible on the side walls of the syringe barrel prior to injection.**
- **Do not use compound if the date has exceeded the expiration date.**
- **Exposure to direct light for more than 5 minutes should be avoided.**

Eliminate air bubbles, if present, prior to injection. Discard solution if solid particles are visible on side walls of the syringe barrel.

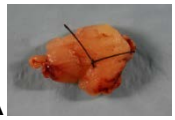
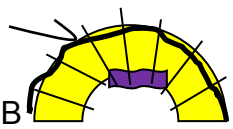
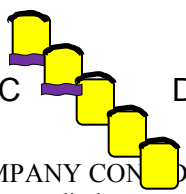
Appendix B: Breast Specimen Pathology Evaluation for the Feasibility Study with the LUM 2.6 Imaging System

1. Final Shaved Margins - MGH Pathology Breast Description of Workflow

- Breast tissue is excised by the surgeon and removed from the patient.
- Orientation sutures for pertinent margins are placed on the specimen by the surgeon.
- The tissue's fluorescent properties are examined by the surgeon or their delegate OR study staff using the Lumicell device.
- The tissue foci with increased fluorescence as observed with the Lumicell device is patteed lightly with non-sterile gauze to remove excess blood or tissue fluid, then a small amount of purple ink is applied on the specimen by cotton swab (the color purple was chosen because this color is not otherwise used by pathology). A small amount of 5% acetic acid is applied to prevent the ink from seeping into tissue crevices.
  The schematic illustrates such a breast margin.



- The specimen is placed in a container labeled with patient information and site.
- The specimen container(s) are carried from the OR to the frozen section lab of the surgical pathology laboratory. The routine yellow surgical requisition sheet is filled out by the surgeon and accompanies the tissue sample.
- Pathology staff are made aware of the Lumicell study; they will prioritize accessioning into the surgical pathology database and provide a surgical pathology number.
- The pathology staff routinely assigned to do the macroscopic examination of the specimen is most likely a pathologist assistant (PA). In some cases, a resident pathologist will handle the macroscopic examination.
- The macroscopic characteristics of the fresh specimen (size, shape, consistency of the tissue, visible or palpable abnormalities) are dictated into the pathology data system. Most breast shaved margin specimens range in size from 1 to 3 cm in largest dimension.

A 
B 
C 
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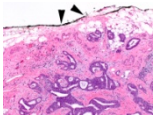
E 

- Images above show the sequence how a breast specimen with suture for orientation (A) is processed in the pathology lab. The schematic indicates a dab of purple ink on the TB side of the specimen and subsequent black ink on the outer surface (M side) carrying the suture (B). The fresh tissue is serially sectioned (C), and pieces placed in a slotted plastic cassette to be immersed in formalin (D). A microscopic overview indicates invasive carcinoma close to the inked margin (arrows).

- The outer surface of the shaved margin specimen that carries a suture and indicates the new final margin as per the surgeon's instructions is now inked black.

- The specimen is serially sectioned along its long axis, perpendicular to the inked surface. Each of these newly obtained fragments is placed in a slotted plastic cassette that is labeled with the surgical pathology case number and consecutive block number (e.g. A1, A2 etc.). The blocks with purple ink will be mentioned in the gross description.

- An example of the gross description may read as follows: "Received fresh, labeled with the patient's name --- and unit number ---, is a 2.5 x 1.8 x 1.2 cm fragment of breast tissue. One surface has a suture which indicates the new margin, as designated by the surgeon. The specimen has a 1 cm area of previously applied purple ink. The surface with the suture is inked black and serially sectioned to reveal yellow-white soft lobulated breast tissue with no lesions grossly identified. The specimen is entirely submitted in cassettes A1-A8, with the purple inked tissue in A3 and A4."

- The tissue is submersed in 10% neutrally buffered formalin and fixed for several hours before routine processing and transfer into paraffin blocks. Sections of 5 µm thickness are cut by the MGH Pathology histology lab, automatically stained with hematoxylin and eosin and coverslipped.

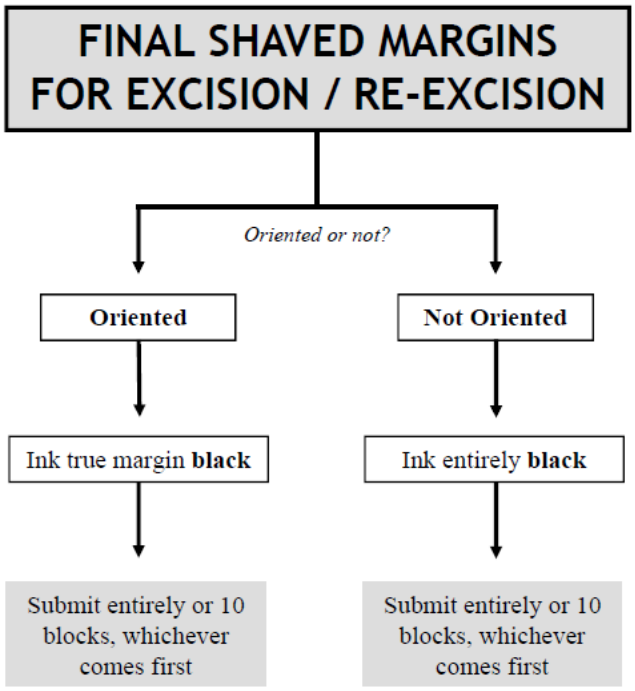
- The H&E stained glass slides are microscopically evaluated by the resident and staff pathologists on service to whom the case is assigned. Occasionally, additional sections or special stains have to be done to arrive at a definitive diagnosis. The final diagnosis is reported and immediately available in the electronic medical record. Ancillary studies (for breast cancer: estrogen and progesterone receptor, and HER2 immunohistochemistry, as well as HER2 fluorescence in-situ hybridization) are usually ordered by the primary pathologist and typically reported as an addendum within the next week.

- Standard grossing procedures as per gross chart are followed throughout the Lumicell study. No additional tissue is submitted if not indicated for clinical / diagnostic reasons.

- Pertinent slides (those with purple ink) will be retrieved from file after all diagnostic work-up is completed by the assigned staff pathologist.

- The study pathologists (EB and/or designate) will evaluate the purple-inked marks applied by Lumicell and correlate with the histopathologic findings.

2. Final Shaved Margins - MGH Pathology Breast Grossing Flowchart



INKING EXEMPTION CRITERIA

Ink all non-cosmetic specimens unless they meet ALL 3 of the following criteria:

1. ***3 or more*** pieces of tissue
2. All pieces approximately ***same size***
3. ***No sutures*** on any of the pieces

Appendix C: Selection of 1.0 mg/kg dose for Phase B

1. Clinical data suggests linear dependency between signal and dose

During Phase A of the Feasibility Study in breast cancer, Lumicell measured the fluorescence signal of tumor and normal tissue in transections of the main lumpectomy guided by a pathologist. The measurements were done in 3 patients at 0 mg/kg (no dose) to determine the autofluorescence signal, and in 5 and 4 patients dosed at 0.5 mg/kg and 1.0 mg/kg, respectively. Results show that signals from tumor and normal tissue increase linearly with dose (Figure 7).

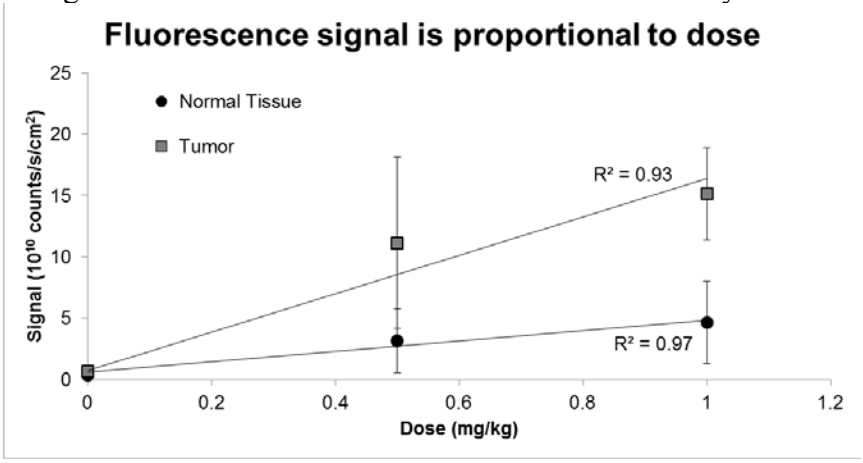


Figure 7: Data from breast cancer patients in Phase A suggest that signal increases linearly with dose for both tumor and normal tissue. Data points show the mean tumor and normal tissue signal at each dose (n=3 at 0 mg/kg, n=5 at 0.5 mg/kg and n=4 at 1.0 mg/kg). Error bars indicate the standard deviation from the mean. Lines indicate a linear fit with the goodness of fit represented by R^2.

1.1 Data suggests decreasing tumor-to-normal tissue signal ratio as dose decreases

We determined the tumor-to-normal tissue signal ratio (T:N, in the Appendix) from the lumpectomy transections and observed that T:N appears to decrease with dose (Figure 8). Based on the observation of linear dependency of signal with dose (Figure 7) and the decrease of T:N with dose, we hypothesize that at 0 mg/kg (no LUM015) T:N corresponds to the contrast from autofluorescence signals between tumor and normal tissue (~2.1). As dose increases, the signal from tumor and normal tissue overcomes autofluorescence signal and T:N approaches the contrast obtained by preferential biodistribution and activation of LUM015 in tumor. We used this hypothesis to build a simple model to predict the behavior of T:N with dose as described in the following section.

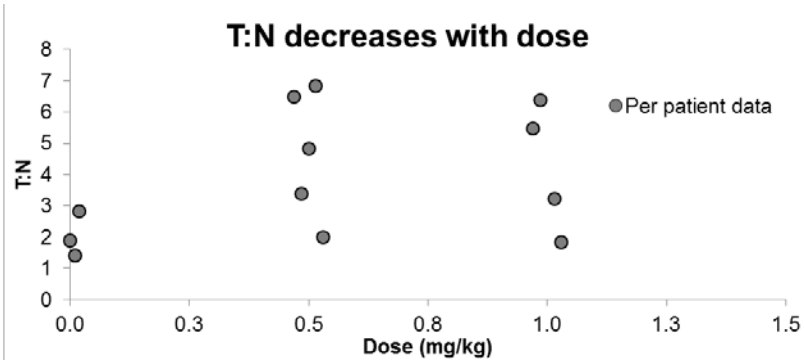


Figure 8: Clinical data from Phase A patients (n=11) suggests that T:N decreases with dose. Each data point represents the mean T:N from one patient.

2. Dose-response model for tumor-to-normal signal ratio

Lumicell has developed a pragmatic model to describe the effect of dose on T:N. The model is based upon the following:

a. A constant background (autofluorescence) from tumor and normal tissue

b. A fluorescence signal for tumor and normal tissue that increases linearly with dose in our dose range.

The model for predicting the mean T:N of a population as a function of dose is given by:

Equation 1

$$T{:}\,N_{model} = \frac{D \times B_T + A_T}{D \times B_N + A_N}$$

where D is dose (in mg/kg), B_T is tumor signal increment per unit dose, A_T is tumor background (autofluorescence) signal, B_N is normal tissue signal increment per unit dose, and A_N is normal tissue background (autofluorescence) signal.
Using the T:N data in Figure 8, we performed a least-squares fit to our model (Equation 1). Results show that our model fits the data (Figure 9). The model predicts that at doses above 1.0 mg/kg, T:N is essentially constant suggesting that higher doses do not improve performance.

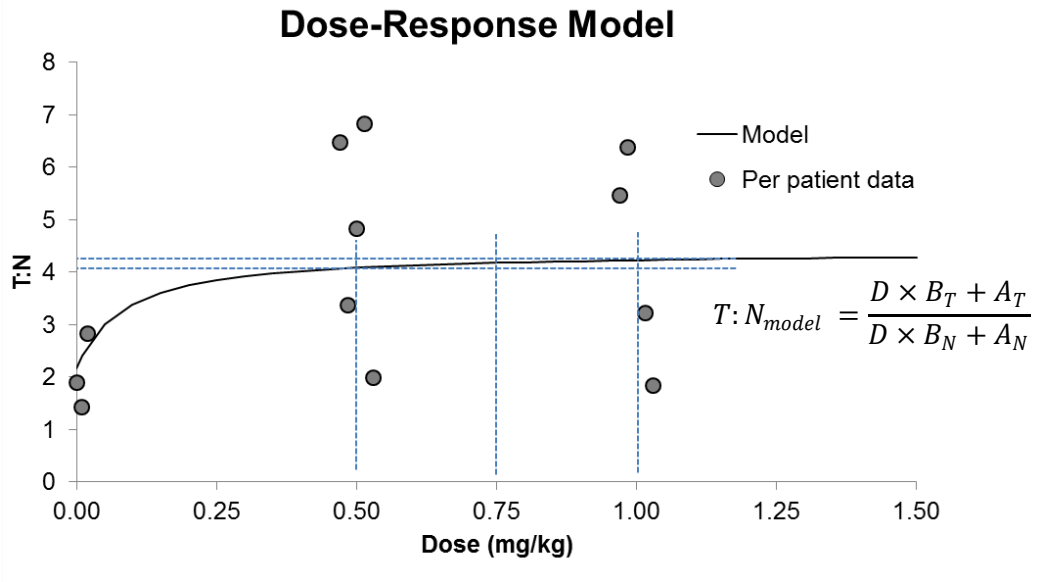


Figure 9: T:N data from Phase A patients (n=11) fit Lumicell's dose-response model (equation 1). Blue dotted lines are plotted to help the reader to note lower expected T:N at 0.5 mg/kg than at 1.0 mg/kg.

3. T:N does not give the whole story: signal variation will dominate at lower doses

Imaging presents unique challenges due to signal variations across the field of view that are not reflected in the dose-response curve for T:N. **Our data show that as dose decreases, the coefficient of variation of tumor and normal tissue signal increases** (Figure 10, see Appendix for definition of coefficient of variation). The large variations in signal result in overlap between tumor and normal tissue signal histograms leading to false positives and false negatives, as observed at 0 mg/kg dose in Phase A.

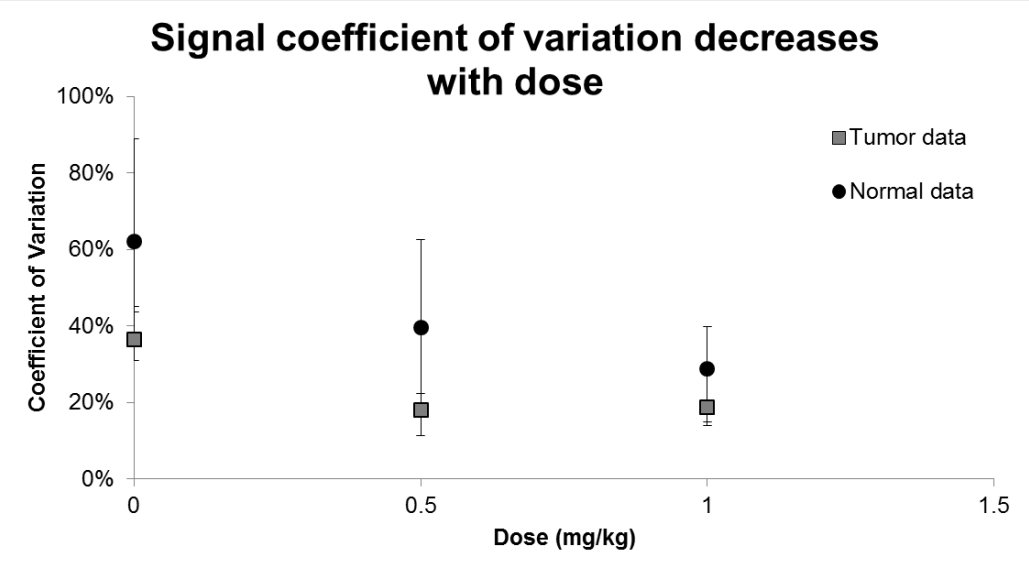


Figure 10: The average coefficient of variation for tumor and normal tissue increases with dose. Data points represent the average coefficient of variation of n=3 patients at 0 mg/kg, n=5 patients at 0.5 mg/kg and n=4 patients at 1.0 mg/kg. The top and bottom error bars indicate the maximum and minimum values of coefficient of variation, respectively.

As mentioned in the conference call, Lumicell is keenly aware that T:N is only a surrogate for the metrics of false positives and false negatives, which are strongly affected by the variations in signal from tissue. These metrics require a larger patient population than a dose response curve of T:N would. To comprehend these effects we have characterized the dependence of variance on dose in the following section, leading to our dose selection for Phase B.

3.1 Model predicts sharp increase of signal variation at doses below 0.5 mg/kg

We used the data from Figure 10 to develop a model to predict the coefficient of variation as a function of dose for both tumor and normal tissue.
The variation in signal includes variations due to instrument noise, autofluorescence heterogeneity, tissue heterogeneity and the uneven distribution of activated LUM015 in tissue. Instead of calculating variance based on these parameters, which are exceedingly difficult to isolate, we used a simple empirical model for estimating the coefficient of variation based on the assumption that the standard deviation of signal increases linearly with dose (see Appendix for data supporting this assumption). The model for coefficient of variation as a function of dose is shown below:

Equation 2
$$CV_i(D) = \frac{C_{1,i} \times D + C_{2,i}}{B_i \times D + A_i}$$

where D is dose (in mg/kg), i represents either tumor (T) or normal tissue (N), and A_i and B_i are the fitted parameters from Equation 1. At low doses, CV_i is dominated by a constant standard deviation independent of dose (represented by $C_{2,1}$.) and the autofluorescence signal (A_i), also independent of dose. At higher doses, CV_i is dominated by the dose dependent components due to increased signal from activated LUM015 in tissue, until it levels off at $C_{1,i}/B_i$. The tumor and normal tissue data were used to fit for the model parameters $C_{1,i}$ and $C_{2,i}$. Our simple model prediction fits our data well in the dose and signal ranges tested as shown in Figure 11.

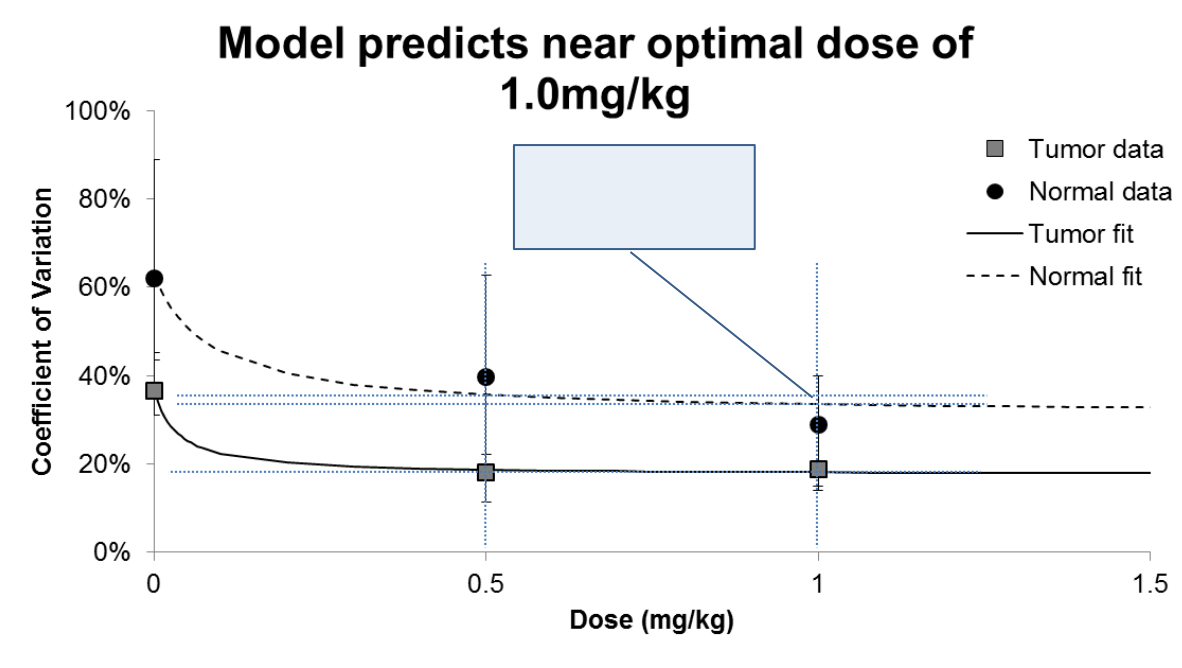


Figure 11: Our coefficient of variation model fits well to the data in Figure 10 and show a sharp increase in coefficient of variation below 0.5 mg/kg. Blue dotted lines are plotted to guide the reader to identify the "near optimal" dose of 1.0 mg/kg based upon the coefficient of variation in normal tissue.

The model in Figure 11 shows a sharp increase in coefficient of variation below a dose of 0.5 mg/kg. This important and critical behavior for selecting a dose is not captured by just measuring the mean T:N. The signal variations observed with auto fluorescence are as large as the signal itself, making the endogenous contrast poor.
Using Figure 11, we estimate that the "near optimal" dose (based upon the coefficient of variation in normal tissue) is 1.0 mg/kg.

4. Dose selection rationale and plan for Phase B

Figure 9 shows the dose-response (T:N) curve falling off below a dose of 0.75 mg/kg and Figure 11 shows the rapid increase in coefficient of variation below 0.5 mg/kg. The combined deterioration of these two metrics below 1.0 mg/kg clearly points to 1.0 mg/kg as the near optimal dose. Selecting a dose lower than 1.0 mg/kg increases the chances of finding false negatives and false positives.
Based on the data and rationale presented above, Lumicell proposes to conduct Phase B of the feasibility study at a dose of 1.0 mg/kg and not to test additional doses in Phase A.

5. Future research

The data from Phase B will be used to design a randomized, multi-center pivotal study that will be statistically powered to evaluate the efficacy of the LUM Imaging System for obtaining negative margins compared to standard of care

Appendix D: Confidentiality and Confirmation Signatures

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