STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: Evaluation of LS301 Uptake in Tumors of Patients Undergoing Partial Mastectomy and Sentinel Lymph Node Biopsy for Breast Cancer

NCT number: NCT02807597

IRB Approved date: 06-06-2023

Evaluation of LS301 Uptake in Tumors of Patients Undergoing Partial Mastectomy and Sentinel Lymph Node Biopsy for Breast Cancer

Phase 1A:
Washington University School of Medicine
Department of Surgery
660 South Euclid Avenue, Campus Box 8109
St. Louis, MO 63110

Phase 1B/Phase 2:
UT Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, TX 75390

Protocol#: 201907135
Version Date: 05/15/2023
STU-2022-0385

Phase 1A:
Principal Investigator:
Julie Margenthaler, M.D.
Phone: (314) 362-7534
Email: jmargenthaler@wustl.edu

Phase 1B & Phase 2:
Principal Investigator:
Deborah Farr, M.D.
UT Southwestern Medical Center
Department of Surgery
5323 Harry Hines Blvd., Dallas, TX 75390
Phone: (214) 648-5870
Email: Deborah.farr@utsouthwestern.edu

Phase 1A:
Co-Principal Investigator:
Samuel Achilefu, PhD
UT Southwestern Medical Center
Department of Biomedical Engineering
5323 Harry Hines Blvd., Dallas, TX 75390
Phone: (314) 362-8599
Email: achilefu@wustl.edu

Phase 1B & Phase 2:
Co-Principal Investigators or Lead Sub-Investigator(s):

Samuel Achilefu, PhD
UT Southwestern Medical Center
Department of Biomedical Engineering
5323 Harry Hines Blvd., Dallas, TX 75390
Phone: 214-645-9900
Email: Samuel.Achilefu@UTSouthwestern.edu

Phase 1A:
Sub-Investigator(s):

Suman Mondal, Ph.D.	Radiology
Christine O'Brien, Ph.D.	Radiology
Rui Tang, Ph.D.	Radiology
Xiao Xu, Ph.D.	Radiology
Jingqin (Rosy) Luo, Ph.D.	Biostatistics
Timothy Eberlein, M.D.	Surgical Oncology
William E. Gillanders, M.D.	Surgical Oncology
Rebecca L. Aft, M.D., Ph.D.	Surgical Oncology
Katherine Glover-Collins, M.D., Ph.D.	Surgical Oncology
Deborah Veis (Novack), M.D., Ph.D.	Pathology

Phase 1B & Phase 2:
Sub-Investigator(s): N/A

Biostatistician:

MinJae Lee, PhD
UT Southwestern Medical Center
5323 Harry Hines Blvd., Dallas, TX 75390
Phone: (214) 648-3803
Fax: (214) 648-3934
MinJae.Lee@UTSouthwestern.edu

IND Number: 131353

NCT Number: NCT02807597

Signature Page

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Amendment/Version #_____

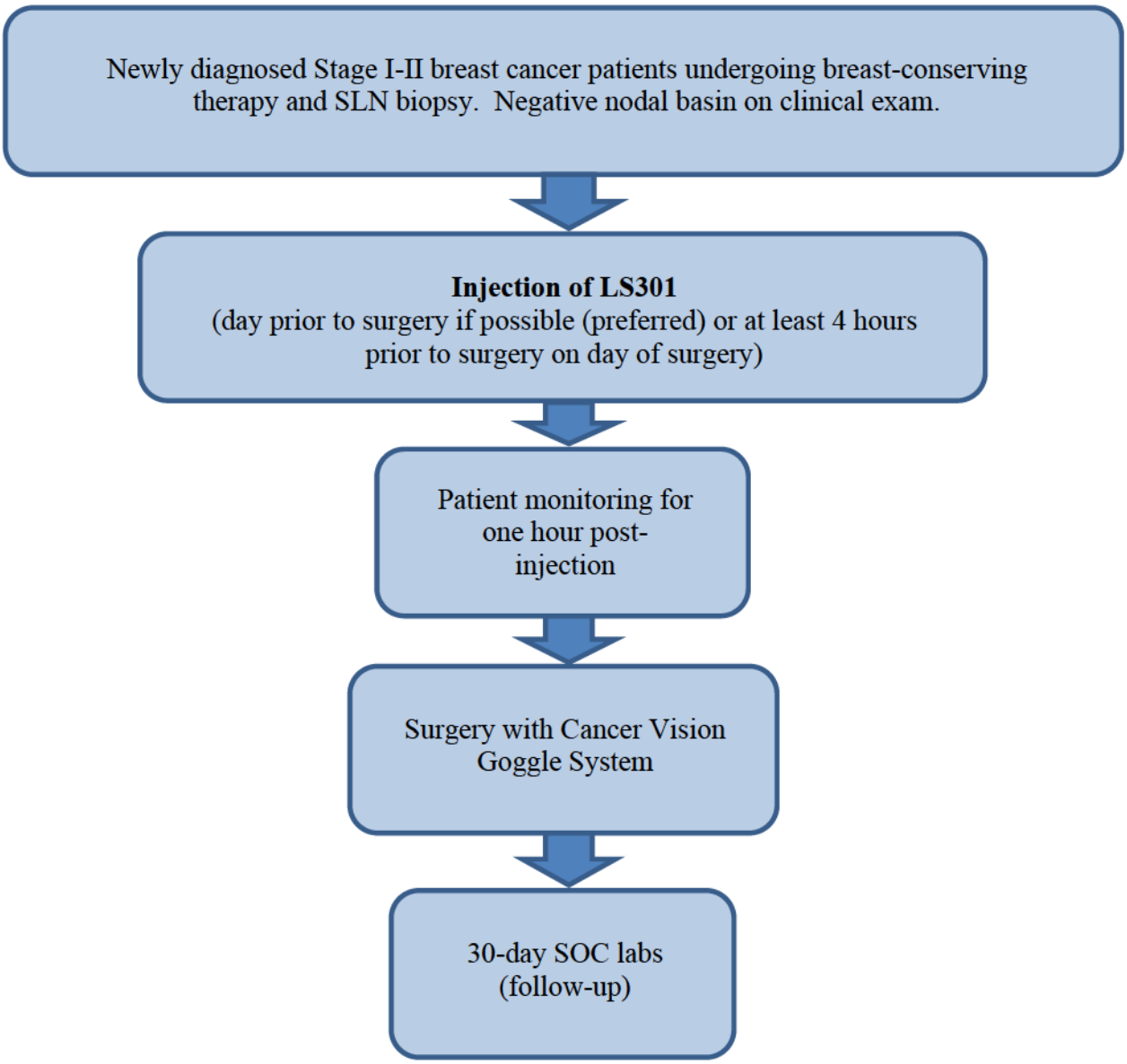
STU-2022-0385
Evaluation of LS301 Uptake in Tumors of Patients Undergoing Partial Mastectomy and Sentinel Lymph Node Biopsy for Breast Cancer

Principal Investigator (PI) Name: **_____**

PI Signature: _____

Date: _____

SCHEMA



LS301 Dose Level	LS301 Dose
Level 1 (starting dose)	0.05 mg/kg
Level 2	0.075 mg/kg
Level 3	0.1 mg/kg

Phase 1 will be complete after establishing the safety and determining the optimal imaging dose of LS301. Enrollment to phase 2 will only begin after the FDA has reviewed and approved the phase 1 data.

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 Surgical Resection of Breast Cancer and Sentinel Lymph Node Biopsy

Surgical resection is the standard of care for many solid tumors such as breast cancer, and sentinel lymph node (SLN) biopsy is used for cancer staging[1]. Before breast surgery, standard imaging methods including mammography and ultrasound are used to identify tumor location. However, these preoperative imaging methods are not able to provide real-time image guidance in the operating room to ensure complete removal of tumors or assess surgical margins. Unfortunately, incomplete tumor removal increases the chances of cancer recurrence by two-fold[2] and necessitates repeat surgery, whereas inaccurate SLN identification may misdiagnose the cancer stage. Despite recent advances in pre-operative imaging methods, surgeons rely on visual inspection, palpation, and tactile evaluation to distinguish cancer from uninvolved tissue intraoperatively, leading to subjective decision-making and variable outcomes. For example, up to 70%[3-5] of patients undergoing breast-conserving reconstructive surgery (BCS) have positive margins, depending on where the surgery was performed, experience of the surgeon, and the tumor characteristics[6-8]. For these reasons, we seek to develop an image-guided method for differentiating tumors from surrounding tissues and offer real-time surgical navigation[10,11].

Similarly, assessment of the SLN for the presence of cancer cells is standard of care for staging breast cancer[1,12,13]. Inaccurate SLN removal often requires additional surgical interventions[14]. Conventionally, patients are injected peritumorally with ^{99m}Tc sulfur-colloid or a new CD206 receptor-targeted radiopharmaceutical, ^{99m}Tc Tilmanocept and a visible lymphotropic blue dye. A handheld gamma-counter is used to localize the region of highest radioactivity, and the blue dye can be used to visualize the SLN. However, radioactive SLN tracking exposes patients and health professionals to ionizing radiation without a direct SLN visualization capability. Although blue dyes can be visualized with the naked eye, SLN tracking by this method is limited to inspection of only superficial lymph nodes and requires a high dose of the dye, which may lead to harmful side effects[15,16]. Without knowing which lymph nodes are positive, multiple nodes are removed randomly for subsequent histologic assessment. The above challenges have spurred interest in developing methods for accurate intraoperative detection of tumors and SLNs in real-time.

1.2 Image-Guided Near-Infrared Fluorescence Systems for Intraoperative Cancer Imaging

The primary goal of image guidance in the operating room is to provide the surgeon with accurate, real-time information about the precise location and boundary of tumors. This will also allow the surgeon to explore alternative treatment planning based on the aggressiveness of the tumor. Advanced instruments that mimic global positioning systems have also been developed, where a preoperative CT or MR image can be projected onto the appropriate anatomical structures. These systems suffer from the limitations of the pre-operative imaging method, unsatisfactory registration due to tissue deformation and motion, and lack of the ability to intraoperatively interrogate surgical margins for the

presence of tumors.

Recent advances in biophotonics and semiconductor technologies have accelerated the emergence of optical imaging as a paradigm-shifting method for real-time cancer detection. Optical imaging can detect biological events ranging from molecular and sub-cellular levels to the level of entire organ systems. In the near infrared (NIR) region between 700 and 900 nm, absorption by intrinsic photoactive biomolecules is low, which minimizes tissue autofluorescence and facilitates thick tissue assessment[21]. Unlike nuclear methods, optical imaging utilizes nonionizing radiation. This explains the recent surge in interest in applying the method in intraoperative procedures, where tumor boundaries, lymph node assessment, treatment response, and important physiological parameters can be monitored in real-time. To date, various optical technologies such as fluorescence imaging have been applied to assess tumor margins[22-31]. Nearly all the systems utilize the NIR light, which can detect fluorescent objects up to 1 cm deep in solid tissue and 5 cm in lung tissue. These optical imaging devices offer high-resolution (typically 125 – 625 μm) and large fields-of-view (7-15 cm).

To date, several NIR-fluorescence image-guided surgery (NIR-FIGS) systems have been developed and successfully used for intraoperative tumor imaging and SLN mapping (SLNM), including FLARE[32], Fluobeam[33], SPY[34], Palomar, and Hamamatsu PDE[35]. The Spy™ system from Novadaq uses an NIR laser to excite the surgical field. The Spy™ system has already been granted three separate 510(k) approvals.

1.3 Wearable Cancer Vision Goggles for Surgical Resection of Cancer

Most of the current optical imaging systems used in the NIR-FIGS have major shortcomings that impede the rapid adoption and seamless integration into the workflow of oncologic surgery. These limitations include the use of bulky hardware, potentially disruptive information display on a remote monitor, mismatch between the system's and surgeon's field of view (FOV), additional effort from another surgical staff to control the device, high cost, and complex instrumentation. To overcome these limitations, our team recently developed and tested in cancer patients a new head-mounted imaging system, Cancer Vision Goggles (CVG), for use in the operating room[36-43]. The schematics and prototypes of the CVG system are shown in **Figures 1** and **2.**

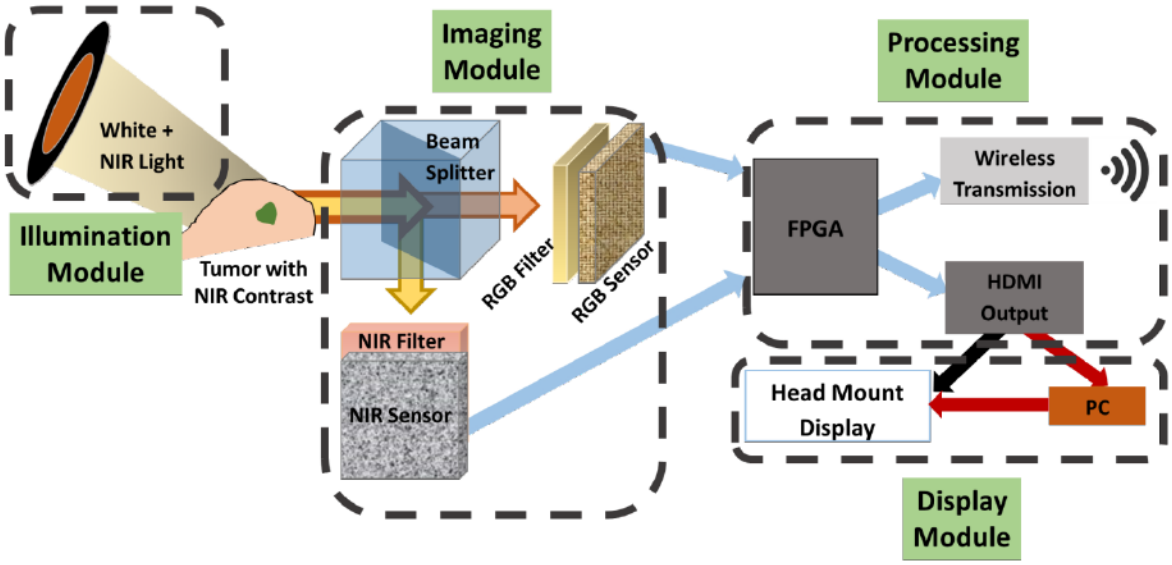


Figure 1: Schematics of the Cancer Vision Goggles system. FPGA - field-programmable gate array; HDMI - high-definition multimedia interface; NIR – near-infrared light; RGB – red, blue, green color space; PC – processing computer unit[43].

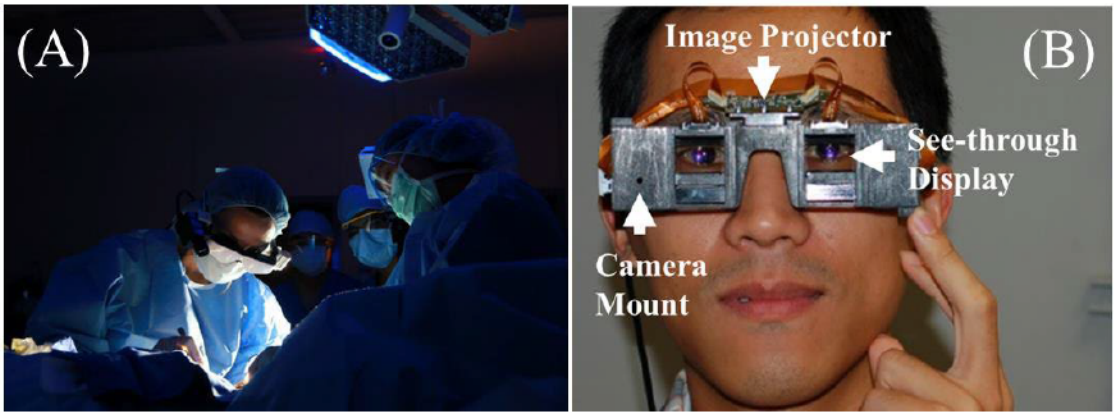


Figure 2: Prototypes of the Cancer Vision Goggles systems. (a) Video-see-through version worn by Dr. Julie Margenthaler in the operating room. (B) Optical-see-through version worn by a researcher. Both systems have been used in the operating room for breast-conserving surgery by Dr. Margenthaler[43].

The CVG holds great promise to overcome the challenges of the current NIR-FIGS systems and expand fluorescence-guided surgery into advanced clinical centers as well as underserved areas, especially those in low-resource settings. This simple goggle-based device is affordable, compact, wearable, and allows hands-free surgical operation. With this device, functional information provided by the NIR fluorescence of contrast agents in cancer cells or the tumor environment can be directly displayed with adjustable amplification on the goggles' eyepiece. The CVG system projects both NIR fluorescence from tumors and the natural color images of tissue onto a head mounted display without latency[43].

1.4 Human Pilot Studies Using Wearable CVG

Clinical feasibility was demonstrated in 15 breast cancer and 5 melanoma patients during SLN biopsy after lumpectomy or mastectomy for breast or wide excision surgeries for melanoma. Indocyanine green (ICG), an FDA-approved NIR fluorescent dye, was employed as the imaging agent. Surgeons used the imaging system comfortably with minimal disruption to the surgical workflow. The CVG allowed clear visualization of 30 SLNs from 10 breast cancer and 5 melanoma patients. Using histologic analysis as the gold standard, the CVG had a detection sensitivity of 100% in comparison to 92.86% ±17.5% for the blue dye and 96.43% ±12.9% for radioactive tracking. In one melanoma patient (Fig. 6), blue dye did not identify two deep-seated SLNs. Similarly, in one breast cancer patient, initial visual inspection did not reveal the SLN and in another patient, radioactive tracking was unable to identify two SLNs. In these cases, the SLNs were clearly identified by CVG. Although the imaging depth with reasonable resolution is about 5 mm, high fluorescence signal from deep-seated SLNs is readily projected to the surface, allowing visualization of SLNs at >5 mm deep after deflection of the overlying tissue layer. This pilot study demonstrates the potential clinical utility of the system for rapid identification of SLNs during surgery.

By FDA guidelines, the CVG device is considered a "non-significant risk" (NSR) device and does not come into direct contact with patient tissue during surgery. We will use these systems to assess the NIR fluorescence of tumor-targeting optical contrast agents in the planned study.

1.5 NIR Fluorescent Molecular Probes for the Accurate Identification of Diverse and Heterogeneous Tumors using NIR-FIGS Is Lacking

Most NIR-FIGS systems are designed to operate in the NIR wavelengths. Successful implementation of real-time NIR-FIGS for more accurate tumor resection requires the development of NIR fluorescent imaging agents that can selectively bind or accumulate in heterogeneous cancer cells, independent of tumor type. Clearly, the development of accurate and broad cancer-specific imaging agents will accelerate clinical translation and use of optical imaging methods in clinics. Optical molecular imaging has evolved from a predominantly preclinical imaging technology to increasing applications in clinics. The promise of high detection sensitivity and real-time imaging feedback has resulted in the development of a myriad of optical molecular imaging probes for cancer application, ranging from receptor-mediated to enzyme activatable molecular probes.[44,45] Notable examples include the use of fluorescent imaging agents in lymph node mapping, image-guided surgery, ophthalmologic applications, and evaluation of lymphatic flow. Researchers are actively pursuing two major reporting strategies in developing molecular imaging probes for human use. First, affinity-based molecular probes are contrast agents that selectively accumulate in tumor tissue relative to the surrounding normal tissue by binding to over-expressed proteins or through other uptake mechanisms. Affinity probes typically involve the conjugation of a fluorescent dye to tumor-targeting biomolecules, such as monoclonal antibodies or high affinity peptide ligands. However, this method is complicated by intrinsic expression of targeted proteins in healthy tissues, tumor

heterogeneity, and patient-dependent variability.[46-53] A second approach is to use activatable fluorescent molecular probes. These molecular probes are designed to have low fluorescence yield until they encounter a molecular target (e.g. enzyme activatable probes)[54] or localize in favorable physiological medium (e.g. pH activatable probes).[55,56] Although these probes have low background fluorescence, the polymeric materials used for their development results in very slow fluorescence enhancement, requiring several hours for optimal signal enhancement in tumors, and may be complicated by non-specific activation. Further, systemic administration of such macromolecules can elicit reactions in patients, a risk that may limit their use.

What is needed to harness the full potential of optical imaging (simplicity, unparalleled detection sensitivity, and real-time feedback) is the development of a NIR molecular probe that can selectively accumulate in cancer cells with high specificity, and a molecule with wide applications similar to 2-deoxy-2-[fluorine-18]fluoro- D-glucose (^{18}F-FDG), which revolutionized cancer diagnosis through the use of PET.

1.6 NIR Fluorescent Molecular Probe, LS301, Selectively Accumulates in Breast Cancer and Other Malignancies

We have championed the development of diverse NIR fluorescent dye-labeled peptides (affinity probes) for use in the molecular optical imaging of tumors, as evidenced by the first demonstration of this approach in rodents[57,58]. As noted above, cancer heterogeneity, even within the same cancer patient, confines the use of these imaging agents to only specific cancer sub-types that overexpress the target biological biomarkers. We have now discovered a new molecular probe, LS301, which selectively accumulates in primary breast and other cancers (especially at the tumor periphery), systemic metastases and metastatic lymph nodes.

1.7 Mechanism of LS301 Cancer-Binding Action

LS301 (**Figure 3**) is a small molecule (<1.6 kDa) consisting of a NIR fluorescent dye (cypate)[59,60] and an octapeptide that is cyclized through a disulfide bond. The NIR dye, indocyanine green (ICG), is an FDA-approved dye for use in human subjects. The spectral properties of ICG are suitable for NIR-FIGS (excitation/emission 785/810 nm in serum and a solution of 20% DMSO in an aqueous medium; molar absorptivity, ε, 240,000 $M^{-1}.cm^{-1}$; and fluorescence quantum yield, ψ, 10%). This dye has been employed to demonstrate the feasibility of using optical imaging methods to study human pathophysiological conditions. Unfortunately, ICG is not tumor-selective and its inability to react with biomolecules has prevented its use for labeling tumor-targeting biological carriers[61,62]. To address this need, we developed an ICG derivative, cypate, which has similar biological clearance profile and spectral properties (absorption, emission, quantum yield) to ICG. Cypate binds reversibly to the hydrophobic pocket of albumin,[63,64] a source of nitrogen and energy for tumors.[65-67] Unlike covalently dye-labeled albumin molecular probes, cypate is released in tumors under mild acidic conditions as it traffics through the endosomal pathway. The released cypate is only transiently retained in tumors before efflux, limiting the tumor-to-background contrast.

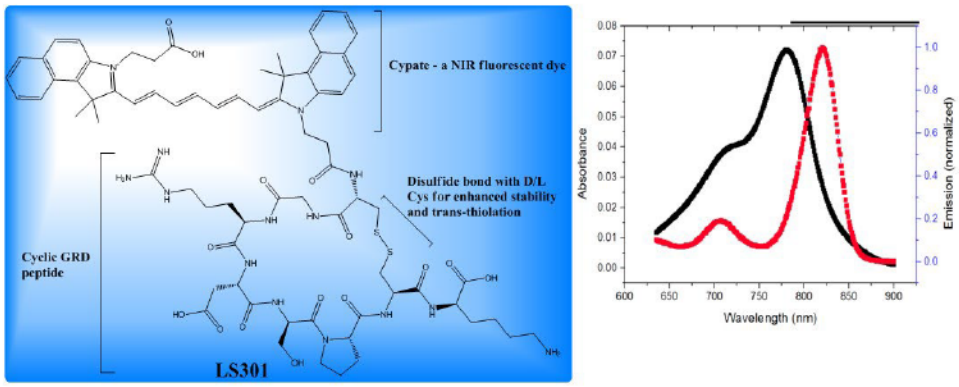


Figure 3: Structure (Left) and spectral properties of LS301 (Right).

To improve tumor retention, we explored using peptides to trap the internalized cypate. The highly reducing environment of tumor cells is known to reduce disulfide bonds to thiols, which can trans-thiolate with cysteine-containing intracellular proteins and trap the molecular probe in cancer cells. Therefore, we conjugated a variety of disulfide-containing peptides to cypate and screened the compounds in immortalized human cancer cell lines and mice with diverse cancer xenografts. Our results showed that LS301 was selectively retained in tumor tissues compared to the other peptide conjugates. Structure-tumor retention analysis revealed that the unnatural D-cysteine linked to cypate confers high biological stability on the molecular probe because of its resistance to rapid degradation by proteases.

NIR fluorescence microscopy of LS301 in diverse tumor cells showed punctate intracellular fluorescence typical of receptor-mediated endocytosis (**Figure 4**). Western blot, proteomic analysis, and blocking studies in cells with diverse inhibitors of albumin endocytosis point to an initial albumin-mediated endocytosis. This mechanism of uptake was further supported *in vitro* and *in vivo* by co-incubation of Alexa 680 labeled bovine serum albumin (BSA) with LS301, which showed co-localization of the two fluorophores in cancer cells at early time points. Divergence of the dye signals was observed at later time-points, indicating the efflux of the Alexa 680 from the cancer cells, while LS301 fluorescence was retained. Calcium also accelerates internalization of LS301 in cancer cells and additional cell studies indicate that one of the intracellular proteins that bind this molecular probe is phosphorylated Annexin A2 (pAnxA2), an indicator of the chronic inflammatory environment of tumors. Based on the ongoing studies, part of the mechanism of LS301 uptake and retention in cancer cells involves albumin-facilitated transport to tumors, followed by binding to pAnxA2 on tumor cells and tumor-associated stromal cells. The complex traffics into the cells where LS301 can be further stabilized via trans-thiolation of the D-cysteine with intracellular proteins under the highly reducing environment of cancer cells. The peptide is eventually degraded and cleared from the body and the dye is cleared via hepatobiliary excretion pathway similar to ICG.

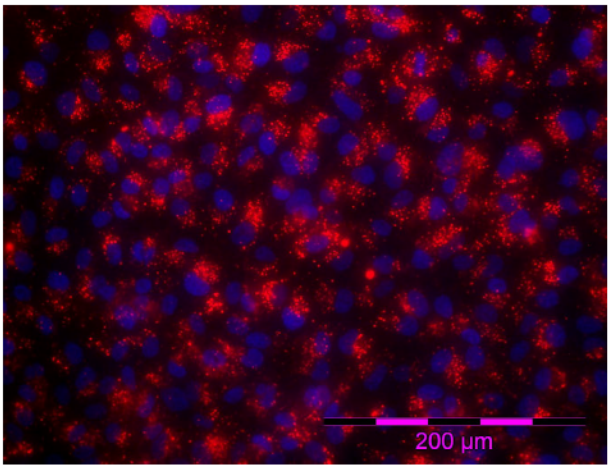


Figure 4: Internalization of LS301 in breast cancer (4T1cells. Red: LS30; Blue: nuclear stain (DAPI).

1.8 LS301 Highlights Tumors in Diverse Animal Models of Cancer

LS301 is produced at the Optical Radiology Laboratory at Washington University School of Medicine in conformance with Good Laboratory Practices as described in CFR21 Parts 210 and 211. We have evaluated LS301 in diverse tumor models in mice, including breast, lung, colon, liver, prostate, pancreatic, and brain tumors. In all cases, LS301 was selectively retained in only the tumor tissue for all different tumor types (**Figure 5**).

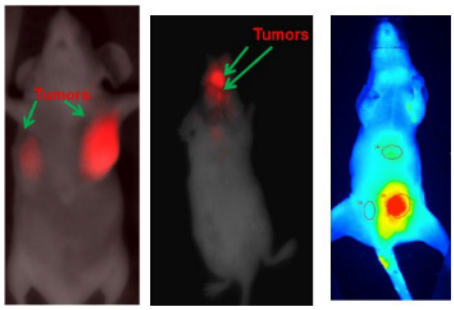


Figure 5: Representative planar images of tumor models that selectively retain LS301. Orthotopic 4T1luc murine (Left), DBT brain (Middle), and HT29 colon (Right) cancer models. False color for LS301 fluorescence is red in the first two panels and rainbow in the last panel with blue as lowest intensity and red as highest intensity obtained 24 hours after injection of LS301.

We have also demonstrated that LS301 can distinguish inflammation from colon adenocarcinoma in colitis model of colon cancer, as well as pancreatitis from pancreatic cancer. The imaging agent also identifies spontaneous microscopic breast tumors or disseminated cervical tumors with high accuracy, as well as positive lymph nodes (**Figure 6**).

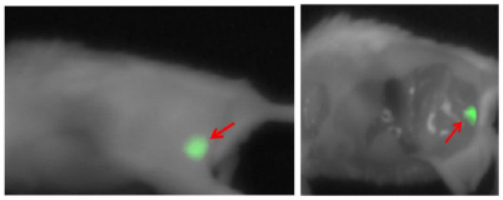


Figure 6: LS301 identifies lymph nodes infiltrated by metastatic breast cancer cells. Preoperative (Left) and after removing the skin. Green: LS301 fluorescence in positive lymph nodes.

To simulate human breast cancer application, we tested LS301 in triple-negative breast cancer using the clinically-relevant patient-derived xenograft (PDX) models (**Figure 7**).

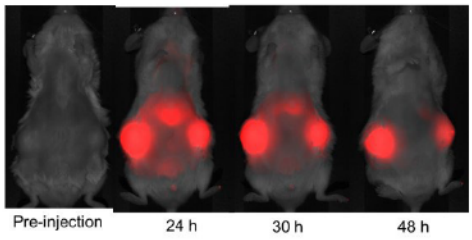


Figure 7: LS301 uptake in PDX breast cancer model. Red: LS301 fluorescence.

1.8.1 Preliminary (Non-GLP) Animal Toxicity Testing in Mice

Potential toxicity of LS301 has been tested in 60 young adult male and female CD-1 IGS mice with an average body weight of 20 g. Twenty males and 20 females were each administered a single dose of 7.5 mg/kg LS301 (150 µg in 100 µL saline containing 1% murine serum albumin) intravenously via the lateral tail vein. Ten controls of each gender were administered a single dose of 100 µL saline. Ten male and 10 female drug-treated animals and 5 male and 5 female control animals were euthanized 2 days post-LS301 administration. The remaining animals were euthanized 14 days post-LS301 administration.

There was no incidence of unexpected death. All animals survived and remained healthy until the planned end points. No clinical abnormalities were noted in any of the animals. There were no differences between the treated and control groups for all measured hematology parameters for both the Day 2 and Day 14 time points. The sodium and chloride levels of treated male animals evaluated on Day 2 were slightly lower than those of the comparable control animals, but were normal by Day 14. There were no abnormal gross necropsy findings attributable to LS301.

We found that microgranulomata formed around golden-brown foci of pigment in the lungs. This finding was observed in 16 of the 20 treated animals evaluated on Day 2, with both genders similarly affected, and was not found in any control animals. Some of these foci were found within vessel lumens, but no further than

the alveolar wall. These lesions were absent in the Day 14 animals, suggesting resolution of this lesions within the 2-week time frame.

1.8.2 Preliminary (Non-GLP) Toxicity Testing in Canines

Three 4-month-old female dogs were treated with 0.9 mg/kg LS301 intravenously over 5 minutes. Animals were monitored closely for the first hour for changes in behavior, respiration, heart rate, and blood pressure, then again at 4, 24 and 48 hours post injection (see attached toxicity report). Blood was drawn for hematology and clinical chemistries before (baseline) and at 1, 4, 24, 48 and 7 days after injection. One of the dogs vomited soon after infusion without any other adverse effects. All animals behaved normally when released and continued normal behavior throughout the duration of the trial.

The whole blood was centrifuged at 3000 x g for 10 minutes and the plasma was separated from red blood cells with pipettes. The plasma samples at different time point were measured on Beckman Coulter DU 640 spectrophotometer directly using un-treated plasma samples as baselines (**Figure 8**). The concentrations of the probe were calculated using the Beer–Lambert law and the extinction coefficient of the probe LS301. One-phase exponential fitting was performed to determine the half-life.

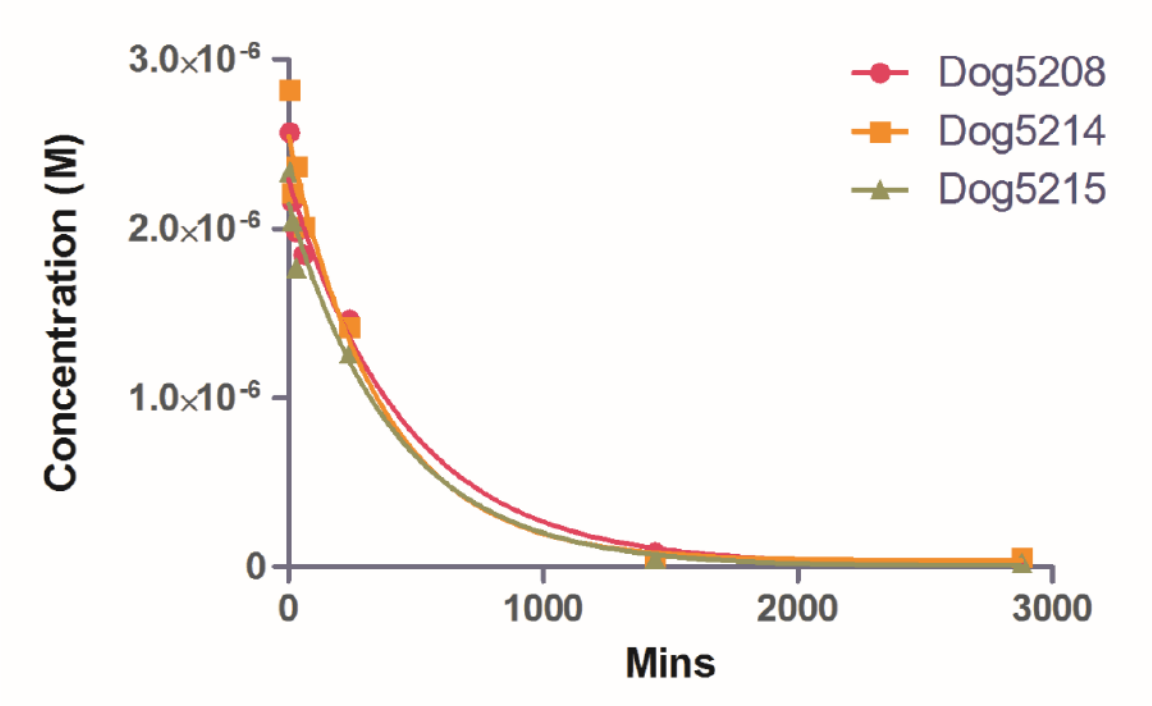


Figure 8: LS301 concentrations in serial plasma samples determined by absorption spectroscopy with pre-injection plasma values as baseline. One-phase exponential least-squares fitting determined an average elimination half-life of 4.8 +/- 0.5 hour (**Table 1**).

Table 1: Calculated blood clearance parameters for LS301 in dogs.

Parameter	Dog5208	Dog5214	Dog5215
K	0.002190	0.002745	0.002378
Half Life	316.5	252.5	291.5
R^2	0.9735	0.9754	0.9796

One of the primary findings was noted in the peripheral blood of dog 5214 in the initial post-treatment period. This animal showed a drop in neutrophils from baseline levels over the course of 5-60 minutes post-drug administration, with recovery of these neutrophil numbers 4 hours post-treatment. This change was notable, and may have suggested either margination of neutrophils, or a shift of neutrophils to the organs. Based on our consultation with a veterinary clinical pathologist, these findings may result from either endotoxemia or idiosyncratic drug reaction; platelets may follow a same pattern as the neutrophils. The other animals did not show a similar pattern in these numbers. Given endotoxemia would be expected to have affected all animals, this finding was presumed to reflect an idiosyncratic drug reaction in dog 5214.

Another finding in the peripheral blood that was noted in all three animals was a slight elevation in creatinine levels at the 4 hour and 24 hour time points (this analyte was not measured at earlier time points). Following further investigation, it was determined that this likely reflected an interaction of the test compound with picric acid used as part of the test methodology for measuring creatinine, forming a chromogen that mimicked the absorbance expected for creatinine.

Aside from the pulmonary congestion and edema already discussed above, the primary findings in the lungs included minimal to mild small foci of pneumonitis. These were of limited distribution. The cause and significance of these, along with the small foci of inflammation noted in the livers of all dogs is not known; however, these are considered common findings in young dogs, and are thought to be related to prior parasitic infection. In particular, infection with Ascarids early in life can produce such lesions. Comparison with control animals on a future study may help clarify this observation. Also noted in all three dogs were small foci of mineralization in the renal papillae. These are very common findings in dogs and were not likely a treatment effect.

1.9 IND-Enabling Preclinical Studies

LS301 was manufactured as required by the Current Good Manufacturing Practice (cGMP) Regulations and used in safety studies recommended by the Agency to support clinical investigation of LS301. An adequately-designed and controlled safety pharmacology study in dogs, an adequately designed single-dose toxicity study in rats, and a bacterial mutagenicity test (Ames assay) were conducted in compliance with GLP regulations, and the study reports contain all required information for GLP compliance. The test systems used in the studies were appropriate and the study designs included appropriate controls, and used appropriate/validated analytical methods. Details of these studies are included in

the revised submission (SN 0001).

1.10 Clinical Study Plan

The primary goal of this human pilot study is to translate the novel imaging agent LS301, which selectively identifies breast cancer cells with high accuracy, to human patients. Diverse animal models of breast cancer have been investigated to surgical removal of spontaneous, disseminated, and diffuse tumors, which were detected to single cancer cell level by LS301. We have also shown that that the NIR fluorescence can be captured efficiently using CVG.

The maximum recommended starting dose (MRSD) for this clinical trial was based on the NOAEL from animal studies. As shown in the GLP toxicology document, the NOAEL from the rat toxicity study is 10 mg/kg for females and 0.5 mg/kg for male rats, which is equivalent to human equivalent dose (HED) of 1.6 mg/kg and 0.081 mg/kg, respectively. Applying a safety margin (typically 10-fold) to the NOAEL gives MRSD of 0.16 mg/kg based on female NOAEL and 0.0081 mg/kg based on the NOAEL in male rats. The highest non-severely toxic dose (HNSTD) in the rat toxicity study was determined to be 10 mg/kg (HED 1.6 mg/kg) in both male and female rats. Another approach that is considered appropriate to determine a safe starting dose is using $1/6^{th}$ of the HNSTD in a more sensitive species. Based on the NOAEL and HNSTD from the rat toxicity study, the maximum recommended safe dose to test in the FIH study was estimated to be between 0.16 and 0.26 mg/kg. While previous investigations with LS301 in mice showed the lowest detectable dose with the CVG to be 0.075 mg/kg (equivalent to HED of that is 0.006 mg/kg), an anticipated optimal starting dose of 0.1 mg/kg will be used as the starting dose in the clinical study. This dose is well within the estimated range for the MRSD estimated from the rat toxicity study.

We propose a phase 1 rolling six dose-escalating design given that this is the first-in-human use of LS301, followed by a phase 2 trial for preliminary evaluation of its diagnostic capabilities (including sensitivity and specificity). During phase 1, LS301 will be administered intravenously to a cohort of six patients at a dose of 0.05 mg/kg; if fewer than 2 DLTs are observed, LS301 will be given at the next high dose level of 0.075 mg/kg; if fewer than 2 DLTs are observed, LS301 will be given at the expected optimal dose of 0.1 mg/kg. Once safety is confirmed and the optimal imaging dose is established, an additional 9 patients will be enrolled in the phase 1 expansion cohort to evaluate for safety.

After phase 1 data has been submitted to and discussed with the FDA, an additional cohort of 88 patients will be enrolled to the phase 2 portion of the trial to assess the diagnostic capabilities of LS301 for identification of positive margins at surgery. Based on preclinical studies[36,37,40,43,68], the anticipated optimal dose for LS301 is 0.1 mg/kg body weight (BW). This dose is a fraction of ICG concentration currently approved for human use. LS301 will be formulated in 1% human serum albumin (HSA) in saline and administered intravenously as a slow infusion over 5-10 minutes. Based on LS301 in 1% HSA and assuming a 60 kg adult with 1.6 m^2 surface area and using the conversion factor k_m= 60/1.6= 37 kg/m^2, the equivalent dose is 100 μg/kg×37 kg/m^2 = 3.7 mg/m^2.

In all patients, LS301 will be administered 4-24 hours before lumpectomy. LS301 uptake in primary breast cancer or SLN versus uninvolved tissue will be visualized with the CVG system during surgery by a member (operating or non-operating) of the surgical team. The NIR light source for the CVG is based on light-emitting diodes (LEDs) or laser light operating at 15-20 mW/cm^2 output at tissue, which can deliver sufficient light to biological tissues and induce fluorescence emission to meet the needs of the planned clinical studies. It should be noted that the light source is below the US FDA recommended limit for NIR exposure and ANSI standard. The fluorescence signals will be received by the detector portion of our device. Gain-settings could be easily adjusted during operation to optimize the contrast between high fluorescence areas (tumors) and low fluorescence areas (normal tissues). Real-time fluorescence image will be displayed in the goggle eyepiece as well as on a secondary monitor to facilitate viewing by other members of the surgical team in the OR. Images from the CVG or SPY will not be used to alter the surgical plan in any way.

Fluorescence in the excised tissue at the margins will be determined by quantitative near-infrared fluorescence microscopy. For each patient, at least 4 specimens from excised tissue will be analyzed, including suspected tumor and suspected healthy tissue. De-identified data will be stored on a computer. Stored information will only have coded numbers and not the names of the patients to ensure patience privacy.

1.11 Rationale

Our preclinical data have demonstrated the feasibility of fluorescence-guided tumor resection by our CVG with LS301 in animal models. In this study, we will conduct intraoperative imaging procedures that have minimal interference with ongoing surgery. The undelying hypothesis is that the accurate detection of all cancer cells highlighted by LS301 during surgery will reduce the number of breast cancer patients with margin positivity to less than 5%, compared to the current surgical paradigm of greater than 20%. The pilot study will obtain critical data required to address the larger question of surgical margin assessment in a full phase I clinical trial.

1.12 Potential Contribution

The ability to identify tumors *in vivo* would be a highly useful clinical tool for oncologic surgeons. When utilized in tumor resection, including SLN biopsy, the proposed procedures can readily detect tumor margins, small nodules, residual tumors, and positive SLNs, thereby reducing the incidence of incomplete resection, the size of healthy tissues resected, and the need for revision surgeries. Moreover, the proposed technology can also offer high spatial resolution in real-time without the use of radioactive tracers. In addition to improving healthcare, the approach can potentially shorten OR time to further reduce cost of surgery. Therefore, the LS301-mediated fluorescence imaging of tumors has high potential impact on breast cancer management.

2.0 OBJECTIVES

2.1 Primary Objectives

Phase 1: to determine the safety and optimal imaging dose of LS301 injected in breast cancer patients.

Phase 2: to determine the ability of this novel fluorescence imaging agent to predict the presence of positive margins around partial mastectomy specimens and positive SLNs during surgical therapy for breast cancer.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Newly diagnosed Stage I-II breast cancer patients undergoing breast-conserving therapy and SLN biopsy.
2. Negative nodal basin clinical exam.
3. At least 18 years of age.
4. Able to understand and willing to sign a written informed consent document.

3.2 Exclusion Criteria

1. Contraindications for surgery.
2. Receiving any investigational agents.
3. History of allergic reactions attributed to ICG or other agents used in the study, include known iodide or seafood allergy. We do not expect many of these adverse reactions with LS301 because it is not radioactive and does not possess iodinated counterions.
4. Presence of underlying lung disease.
5. Pregnant. Female patients of childbearing potential must have a negative serum or urine pregnancy test no more than 7 days before start of participation.
6. Breastfeeding. Patients who are breastfeeding are excluded from this study because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with LS301.

3.3 Inclusion of Women and Minorities

Breast cancer is rare in men; therefore, it is likely that only women will enroll in this trial, although men are eligible. People of all races and ethnicities may enroll.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center for phase 1A and UT Southwestern Medical Center Velos database for phase 1B and phase 2.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database for phase 1A and UT Southwestern Medical Center Velos database for phase 1B and phase 2.
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database for phase 1A UT Southwestern Medical Center Velos Database for phase 1B and phase 2.

All patients must be registered through the Siteman Cancer Center OnCore database for phase 1A UT Southwestern Medical Center Velos database for phase 1B and phase 2.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

4.4 Baseline Data Collection

1. Patient demographic information: age, sex, race, ethnicity
2. Patient height, weight, and body mass index (BMI)
3. Patient medical history and concomitant medications

5.0 STUDY PROCEDURES

5.1 LS301 Preparation

LS301 will be prepared under cGMP protocol and supplied in an amber-colored vial as a lyophilized product in 1% HSA/PBS aqueous solution. Each vial will contain 2 mg LS301. Before administration, 10 mL of PBS will be added to the lyophilized powder and mixed by shaking for 1 minute. The aqueous formulation is stable at room temperature for 4 hours or in the refrigerator (2-8°C) for 24 hours. The agent will be administered 4-24 hours before surgery.

5.2 Procedures

5.2.1 Phase 1

A rolling six design (refer to Section 8.1) will be used to enroll patients who are being treated for pathologically proven breast cancer prior to definitive surgical interventions in the phase 1A dose escalating portion of the study, during which LS301 will be administered intravenously at one of three doses (see table below). Patients will be observed for dose-limiting toxicities (DLT) (refer to Section 5.2.1.3).

LS301 Dose Level	LS301 Dose
Level 1 (starting dose)	0.05 mg/kg
Level 2	0.075 mg/kg
Level 3	0.1 mg/kg

The patient will undergo surgery 4-24 hours after administration of LS301 using current best practices. Breast tissue and axillary LNs will be imaged non-invasively prior to start of surgery and excised breast tissue and SLNs will be examined ex vivo for the presence of LS301 fluorescence using our Cancer Vision Goggles (CVG) to determine if LS301 accumulated in the breast cancer. We will quantify fluorescence intensity in the cancer to establish the feasibility of observing LS301 fluorescence with the imaging system.

The dose used in cohort 1 is the lowest dose (0.05mg/kg) and was used exclusively for safety assessment. The investigators did not expect to detect LS301-associated tumor tissue fluorescence before incision at this lowest dose level. Therefore, cohort 1 patients will not be imaged non-invasively to assess tumor tissue fluorescence before incision. Non-invasive tumor fluorescence examination will be initiated from cohort 2 onwards, where higher doses will be used.

Investigators do not expect the 1st dose (0.05 mg/kg) or 2nd dose (0.075 mg/kg) to lead to any detectable fluorescence non-invasively in the axilla before sentinel lymph node excision due to the low concentration of expected LS301 retention in lymph nodes. Therefore, lymph node fluorescence will not be assessed non-invasively before incision for cohorts 1 and 2. Investigators will plan to image lymph node regions non-invasively starting with cohort 3 due to the higher injected dose (0.1 mg/kg).

After the optimal dose has been determined, an additional 9 patients will be enrolled in the phase 1 expansion cohort to evaluate for safety. This expansion cohort is considered phase 1B and will be conducted at UT Southwestern Medical Center.

5.2.1.1 Dose Escalation Schema

LS301 Dose Level	LS301 Dose
Level 1 (starting dose)	0.05 mg/kg
Level 2	0.075 mg/kg
Level 3	0.1 mg/kg

Dose escalation may occur as soon as the last patient in a cohort has completed her one-hour post-injection toxicity monitoring.

5.2.1.2 Definition of Optimal Dose

The optimal dose will be the dose at which fewer than 2 DLTs are observed and optimal image quality is observed. It need not be the maximum tolerated dose if optimal image quality is observed at a lower dose than the MTD.

5.2.1.3 Definition of Dose-Limiting Toxicities

DLT is defined as any grade 2 or above toxicity that occurs during the hour after injection that is considered possibly, probably, or definitely related to LS301.

5.2.1.4 Dose Escalation Criteria

The molecular probe LS301 is an imaging agent binding to breast cancer cells and other malignant cells and will be visualized through by CVG which is a non-significant risk device. Similar to the FDA-approved agent ICG, we do not expect any serious adverse events caused by the agent.

A rolling six design will be used for the phase 1 portion. In the rolling six design, 2 to 6 patients can be enrolled to a dose each time. The dose de-escalation occurs when two or more DLTs occur at a dose level, while dose escalation occurs with 3/3/, 4/4, 5/5, 5/6, or 6/6 patients are evaluated without DLT. (Refer to Section 8.1 for further details.)

5.2.2 Phase 2

After data from the phase 1 portion of the study have been submitted to and reviewed by the FDA, and if analysis of the data from phase 1 confirms the safety of administering LS301 in humans and establishes the optimal imaging dose, 88 patients will be enrolled in the phase 2 portion of the study after any necessary protocol revisions have been made based on phase 1 data and FDA approval.

It is expected that the study procedures for phase 2 will be the same as the procedures in phase 1. The optimal imaging dose of LS301 determined in phase 1 will be administered in phase 2.

The breast tissue and axillary LNs will be imaged non-invasively using the CVG and worn by a non-operating member of the surgical team. After removal of the breast cancer and SLNs using current best practice, a member of the surgical team who is not conducting the surgery will wear the CVG to determine if LS301 fluorescence is present at the margin and in SLNs, using a global threshold of 20% signal above background or a dynamic statistical threshold to indicate a positive margin. The accuracy of determining margin positivity will be established by comparing the CVG assessment to histopathology.

5.2.3 Administration of LS301

The patient will undergo intravenous injection of LS301 4-24 hours prior to surgery. A study nurse or other qualified member of the study team will inject the LS301 via IV over 5-10 minutes in the office or pre-op room (as applicable) and will monitor the patient as described in Section 5.4 below. Administration of LS301 will not interfere with the standard of care, including all standard of care for lymph node assessment using ^{99m}Tc Tilmanocept injection on the day of or day before surgery.

5.2.4 Intraoperative Review

The operating surgeon will conduct breast cancer surgery as usual without using a device to visualize LS301 fluorescence. To prevent bias in data acquisition, a non-operating member of the study team will wear the CVG before the start of surgery to examine the breast tissue and axillary LNs non-invasively and at the completion of the surgery to examine the excised tissue, SLNs and surgical cavity. Findings with the study will be recorded but will not change the standard of care. The operating surgeon will remain blinded to the fluorescence images throughout the operation. The examining surgical team member will wear the CVG and image all six anatomical aspects (superior, inferior, anterior, posterior, medial and lateral) of the surgical specimen as well as the surgical cavity for LS301 fluorescence. The SPY system will be used for confirmatory fluorescence imaging and compared with CVG detection of LS301 fluorescence, during phase 2 of the study.

Detection of LS301 fluorescence at the cut edge of the excised specimen will determine margin positivity and presence of fluorescence in the SLNs indicate presence of metastatic cancer cells. Given that CVG can detect LS301 fluorescence in tumors several millimeters deep, investigators will use pathology data to initially train the system by correlating fluorescence intensity to background ratio with pathologist's margin assessment. The information will then be used in phase 2 to predict margin positivity in the OR. The detection of fluorescence on excised tissue without correlative fluorescence in the tumor cavity after lumpectomy will predict

a negative margin for invasive ductal carcinoma. For DCIS, laser power modulation within the approved limit or dual excitation or emission method will be used to estimate the depth of fluorescence source. In general, a signal to background ratio of 1.2 (20% higher intensity than background) will be used as a global threshold to determine margin and SLN positivity status. In addition, a dynamic statistical thresholding approach will be used to delineate the boundary of tissue fluorescence. An intensity-based heat color map will be used for false color representation of the fluorescence to guide the examining surgeon. Areas with high LS301 accumulation, will be represented as red-hot compared to green and blue for surrounding tissue. A continuum of red fluorescence will indicate that the entire excised tissue is cancer. Fluorescence gradient using the heat color map showing red to blue from the edge of tumor tissue will indicate a positive margin. When the fluorescence emanating from tumors away from the margin (> 2 mm), hazy blue fluorescence will be observed at the surgical margin and will be classified as clear margin.

If this simple approach fails to accurately identify positive or clean margins, we will use a dual wavelength excitation for the depth-profiling of fluorescence emission. It is well known that light travels through tissue differently across wavelengths due to wavelength-dependent optical properties. When a fluorophore is excited using two wavelengths, the intensity falloff with depth of each wavelength will be related to the depth of the fluorophore. Because both wavelengths are exciting the same fluorophore concentration, the ratio of the fluorescence generated yields the depth of the fluorophore irrespective of concentration accumulated in the tumor tissue. The depth of fluorescence in the resected sample will be estimated using two spectrally separated NIR wavelengths to excite LS301 fluorescence. The ratio of the emitted intensities imaged for both excitation wavelengths will be closer to one when the tumor tissue is at the cut surface. At higher depths the ratio will deviate away from 1 as the shorter wavelength excitation is attenuated more than longer excitation. A depth map of tissue fluorescence will be created and compared to histopathology for determining the accuracy of predicting positive or clear margin status and SLN metastatic status. The dual-wavelength method will initially be implemented as a standalone portable closed box configuration which will then be miniaturized and implemented in the CVG.

The surgically removed tumor mass and SLNs will be pathologically examined under standard protocols. At least 4 specimens from the excised tissue of a patient, two suspected tumor and two suspected healthy tissue per patient will be analyzed.

The tumor specimens generated after breadloafing by the surgical pathologist will be individually imaged using the CVG and the dual-wavelength closed box imaging system before fixation and paraffin embedding to record the fluorescence intensity localization and depth profiles. Pathologist will provide tissue samples that will be sectioned and mounted on slides. The sectioned tissue slides will be imaged for LS301 fluorescence using high-resolution confocal microscopes. These will be compared to standard Hematoxylin & Eosin staining of tumor tissue to verify

LS301 specificity for cancer cells. Additional immunohistochemistry will be performed on these tissue sections to verify or identify LS301-associated biomarkers.

5.3 Evaluability

All patients who receive an LS301 injection are evaluable for toxicity. Patients are evaluated from time of injection to one-hour post-injection.

Patients enrolled in phase 1 are evaluable for DLT assessment during the hour after injection. Patients in phase 2 will not be assessed for DLT.

5.4 Safety Evaluation

The safety of the LS301 will be assessed by closely monitoring the patients' vital signs and laboratory tests as described below.

5.4.1 Vital Signs

All vital signs will be recorded on the case report form. Vital signs may be obtained with the subject in the supine or upright position. Care will be taken to obtain subsequent recordings with the subject in the same position (supine or upright). Although allergic or other immediate adverse reactions are not anticipated, subjects will be monitored for at least 60 minutes post injection in an area where emergency equipment is available. Vital signs will be obtained pre-injection (within 30 minutes prior to injection of LS301), within 30 minutes post injection, at 60 minutes post injection, and prior to surgery. Vital signs will include the following: heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, and body temperature. The following changes from baseline will be considered noteworthy:

- Heart rate > 20 beats per minute
- Systolic blood pressure > 20 mm Hg
- Diastolic blood pressure > 10 mm Hg

Noteworthy changes will be documented on the CRF, and they will be marked as clinically significant or not. If clinically significant, the principal investigator will assess the causality of the change to the injection of LS301. Clinically significant changes in vital signs will be followed up hourly until they return to baseline or normal levels, or until follow-up is no longer warranted. If a clinically significant change of a vital sign is noted, it will be reported on the adverse event log.

5.4.2 Clinical Laboratory Testing

Laboratory tests will consist of the following: standard CBC, comprehensive metabolic panel and urinalysis obtained at the following time points:

- Baseline: within 30 minutes prior to injection of LS301
- 60 minutes after injection of LS301
- Approximately one hour prior to surgery

Approximately 14 ml of blood and a urine sample will be collected at each time point. For patients enrolled in phase 1B and phase 2 of the study, a portion of the blood samples from each time point will be used to identify the predominant blood protein(s) that binds LS301 and mediates the bioavailability of the agent (refer to Section 5.6). Phase 1B refers to phase 1 study after completing drug safety and selecting optimal dose for testing at 2 and 4 hours from injection to surgery. The current project is that if 3 patients at each of the 3 dose level do not experience adverse events, we will need only 9 patients for the drug safety study (phase 1A) and additional 9 patients at the selected dose for testing at 2, 4, and >12 hours (3 patients for each time point) from injection to surgery.

The results of standard of care laboratory testing obtained up to 30 days post injection will be reviewed. Analysis of this will take into account that the subject has undergone surgical removal of tumor and neoadjuvant treatment including hormonal therapy or chemotherapy.

The following changes from baseline clinical laboratory values are considered to be noteworthy and require assessment as to clinical significance when they fall outside of normal limits. Clinically significant changes in laboratory values should be followed up daily until they return to baseline or normal levels, or until follow-up is no longer warranted. Laboratory values that are abnormal at baseline but move into normal range will not be considered clinically significant. If a clinically significant change of a laboratory value is noted, it will be reported on the AE log.

Analyte	**Change from baseline**
Hemoglobin	> 2g/dL
WBCs	> 1 K/mm^3
Neutrophils	> 10 %
Lymphocytes	> 10%
Platelets	> 50 K/mm^3
Creatinine	> 0.75 mg/dL
BUN	> 20 mg/dL
Calcium	> 1mg/dL
Sodium	> 5 mmol//L
Potassium	> 0.5 mmol/L
CO_2	> 4 mmol/L
ALT (SGPT)	> 150 IU/L
AST (SGOT)	> 100 IU/L
Alkaline Phosphatase	> 150 IU/L
Total Bilirubin	> 0.5 mg/dL
Albumin	> 1g/dL

Changes in pre and post injection urinalysis will be noted but due to variability will not be used to determine clinical significance for changes due to the injection of LS301.

5.4.3 Electrocardiograms (ECGs)

A standard 12-lead ECG will be obtained on all subjects at baseline (within 30 minutes prior to injection of LS301), 5-10 minutes post injection, and prior to discharge at 60 minutes.

The following table lists criteria for normal limits and clinically notable limits for ECGs in adults.

	Normal Limits (msec)		**Notable Limits (msec)**	
ECG Variables	**Low**	**High**	**Low**	**High**
PR interval	120	200	<120	>200
QRS interval	50	100	< 50	>100
RR interval	600	1000	<600	>1000
QT interval	No lower limit	≥ 460	No lower limit	≥ 460

5.4.4 Identification of Blood half-life of LS301

LS301 half-life will be determined using 500 µL of blood collected at the same time as the CBC and CMP at baseline, 60 minutes post-injection, and one hour before surgery. These blood samples for research use will be drawn into pink top light-protected tubes, brought to the Achilefu lab for analysis, to identify the concentration of LS301 and determine of the half-life of the agent.

5.5 Risks and Adverse Event Monitoring

A potential risk specific to this experiment procedure is the injection of LS301. The imaging agent is not known to cause any serious adverse events in preclinical models. However, it is possible that some patients may experience mild reactions similar to those they might experience related to OctreoScan (a nuclear (SPECT) imaging agent) such as dizziness, fever, changes in liver enzymes, and nausea. The dose escalation procedure will allow us to monitor these reactions from low to high doses of LS301. There are other experimental tumor targeted imaging agents currently being evaluated in human patients (see the table below). Most of them either user antibodies tagged with ICG or dye-labeled biomolecule.

List of imaging agents under clinical investigation for image-guided surgery

Sl no	Agent	Clinical Trial	Trial type	Cancer type	Adverse reactions
1	Cetuximub IRDye800	NCT01987375	Phase 1 dose escalation study	Head and neck	Yes, in humans[69] and monkeys[70]
2	Panitumumab IRDye 800	NCT02415881	Phase 1 safety/ efficacy study	Head and neck	No reports found
3	Bevacizumab-800CW	NCT01508572 NCT02583568	Phase 1 Phase 2	Breast	No reports found. None reported for PET version[71]
4	ICG and goggle	NCT02316795	Pilot	Breast and Melanoma	None reported[43]
5	ICG and Fluobeam	NCT01738217	Phase 1 and Phase 2	Liver	No reports found
6	BLZ 100 and Fluobeam	NCT02097875	Phase 1 dose escalation	Skin cancer	None in humans[72] and dogs[73]
7	BLZ 100 and unspecified system	NCT02234297	Phase 1 safety	Glioma	None in humans[72] and dogs[73]

The risks from the CVG system are extremely low. The system does not come into contact with the test subject. It utilizes safe invisible NIR light between 700 and 900 nm at a very low fluence rate ($\leq$ 25 mW/cm^2). This imaging system meets the US FDA definition of a "non-significant risk" device, and indeed could be considered "equivalent" to the Spy™ system, which has already obtained US FDA approval as a non-significant risk device.

The US FDA limit for NIR exposure is 10,000 mW/cm^2 and the current system utilizes 2.5-25 mW/cm^2 for a total duration of less than 30 minutes in the initial imaging and 10 minutes per subsequent imaging segments. This is equivalent to the current NIR dose emitted from the standard 5-lantern surgical lights commonly used in the operating room.

Patient safety monitoring for adverse events as a result of this study will be conducted as described in Section 5.4 above. Any reportable adverse event will be reported in accordance to Washington University HRPO regulations (see Section 6.0) for phase 1A. Any reportable adverse event in phase 1B/phase 2 will be reported in accordance to UTSW HRPP/IRB guidelines (see Section 6.0).

5.6 Collection of Specimens for Research Analyses (Phase 1B and Phase 2 ONLY)

5.6.1 Identification of Blood Proteins that Bind LS301

Five mL of blood collected at the same time as the CBC and CMP at baseline, 60 minutes post-injection, and one hour before surgery will be used to identify the predominant blood proteins that bind LS301 and mediate the bioavailability of the agent. This research blood will be drawn into a pink top light-protected tube and brought to the Achilefu lab.

5.6.2 Determination of LS301-Associated Biomarkers in Breast Cancer Tissue

After surgical removal of the cancer, the second (non-operating) surgeon will examine the tissue with the goggles and an additional ink will be used to identify any LS301 fluorescence in the surgical margin. This surgeon will then use the goggles to procure cores from the inked and sectioned specimens in surgical pathology. These cores will then be frozen and delivered to the biospecimen core for accessioning, processing, and storage. The specimens will then be analyzed to identify LS301-associated biomarkers.

5.7 Data Submission Schedule

Case report forms with appropriate source documentation will be completed according to the schedule listed in this study.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On Study Form Medical History Form	At the time of study entry
LS301 Injection Form	After injection
Surgery Form	After surgery
Follow-Up Labs Form	30 days after injection
Toxicity Form	Continuous
Death Form	Time of death
MedWatch Form	As described in Section 6.0

5.7.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 6.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac

disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

5.8 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum or urine pregnancy test within 7 days prior to administration of LS301 before inclusion in this study.

5.9 Duration of Study Participation

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue in the protocol, the patient should be removed from the study and the reason for discontinuation documented in the case report forms.

Patients will be on study for the duration of the screening process and the surgery or until one of the following criteria applies:

- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study for phase 1A
- The UTSW Department of Surgery or Simmons Comprehensive Cancer Center to close the study for phase 1B and phase 2

Follow-up will continue for 30 days post-surgery (for collection of follow-up lab data).

6.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix A for definitions and Appendix B for a grid of reporting timelines.

Adverse events will be tracked for one hour following administration of LS301.

- Baseline adverse events, which shall be recorded on the medical history CRF.
- For purposes of this protocol, adverse events collected and documented on CRFs will not include any events related to surgery.

Refer to the data submission schedule in Section 5.7 for instruction on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 6.1a for phase 1A.

Reporting requirements for UT Southwestern Medical Center study team may be found in Section 6.1b for phase 1B and phase 2.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 6.2 for phase 1A.

The UTSW Human Research Protection Program Office (HRPPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 6.2 for phase 1B and phase 2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 6.4. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

For the purposes of this study, AEs related to surgery will not be collected or considered reportable.

6.1 Sponsor-Investigator Reporting Requirements – Washington University

Phase 1A: Washington University

6.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

6.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Sponsor Investigator (or designee) is required to notify QASMC of any unanticipated problems involving risks to participants or others occurring at WU or an BJH or SLCH institution that have been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgement via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

6.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A), no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
  - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within 15 calendar days after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman

Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

6.2 Sponsor-Investigator Reporting Requirements – UT Southwestern

Phase 1B and Phase 2:

6.2.1 Reporting to the Human Research Protection Program office (HRPP) at UT Southwestern Medical Center

Reporting will be conducted in accordance with UT Southwestern Medical Center IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

6.2.2 Reporting to the Data and Safety Monitoring Committee (DSMC) at UT Southwestern Medical Center

For local patients, in addition to the sponsor monitoring, the Simmons Comprehensive Cancer Data Safety and Monitoring Committee (DSMC) will be monitoring only local unanticipated SAE's as reported to the IRB. The chair will review only local unanticipated SAE's in real time. These are then reviewed quarterly at DSMC meetings The chairman of the DSMC is responsible for this ongoing monitoring. Monitoring of serious adverse events happens by the DSMC chairman in real time and an annual review takes place to look at trends in adverse events, accrual and other applicable study events. The Principal Investigator (or a designated co-investigator) will be primarily responsible for monitoring study accrual rate, study attrition/withdrawals/dropouts, patterns of adverse events and/or unanticipated event and patterns of protocol violations and/or deviations at this site.

Trial monitoring will be conducted no less than annually and refers to a regular interval review of trial related activity and documentation which includes but is not limited to accuracy of case report forms, protocol compliance, timeliness and accuracy of Velos entries and AE/SAE management and reporting. Documentation of trial monitoring will be maintained along with other protocol related documents and will be reviewed during internal audit.

The Sponsor Investigator (or designee) is required to notify DSMC of any unanticipated problems involving risks to participants or others occurring at UTSW that have been reported to and acknowledged by HRPP. (Unanticipated problems reported to HRPP and withdrawn during the review process need not be reported to DSMC.)

DSMC must be notified in real-time as they are reported to the IRB to the UTSW Simmons Cancer Center DSMC Coordinator.

6.2.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPP/DSMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A), no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within 15 calendar days after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Department of Surgery Research team or Simmons Cancer Center team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Department of Surgery Research team or Simmons Cancer Center team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

6.3 Exceptions to Expedited Reporting – Washington University and UT Southwestern

Events that do not require expedited reporting as described in Section 6.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

7.0 DATA AND SAFETY MONITORING

7.1 Phase 1A: Washington University

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least one patient has been enrolled) or one year after accrual has opened (if no patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every 6 months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician name
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study

- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

7.2 Phase 1B and Phase 2: UTSW

In compliance with the Simmons Cancer Center Data and Safety Monitoring Committee, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Simmons Cancer Center Data and Safety Monitoring Committee (DSMC) semi-annually beginning six months after accrual has opened (if at least one patient has been enrolled) or one year after accrual has opened (if no patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every 6 months, and provide a semi-annual report to the DSMC. This report will include:

- HRPP protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician name
- Date of initial HRPP approval, date of most recent consent HRPP approval/revision, date of HRPP expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules

- Summary of toxicities separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Study Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Study Coordinator becomes aware of an adverse event, the AE will be reported to the HRPP and DSMC according to institutional guidelines.

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design

A rolling six design which is intended for relatively safe trials and shortening the study duration is proposed for the phase 1 portion [74]. In the rolling six design, three to six patients can be enrolled to a dose each time. The dose de-escalation occurs when two or more DLT occur at a dose level while dose escalation occurs when 3/3, 4/4, 5/5, 5/6 or 6/6 patients are evaluated without DLT [75]. Three dose levels will be tested in the phase 1 study, 0.05, 0.075 and 0.10 mg/kg. LS301 will be administered intravenously at a starting dose of 0.05 mg/kg to a cohort of six patients and escalate/de-escalate following the decision rule of the rolling six design. Once six patients have been included at the current dose level, inclusions are suspended until at least five of the six patients have completed the procedure without DLT observed in the evaluation period. MTD is hit where two or more patients out of six at a dose level experience DLT. We do not expect any serious adverse events related to LS301 at the initial Dose Level 1. Once the MTD is determined, an expansion cohort with 9 patients will be tested at the MTD and an optimal imaging dose will be recommended for the subsequent phase 2 trial.

At the completion of the expansion cohort, a single arm phase 2 trial will be performed at the optimal imaging dose. A breast surgeon will perform breast conserving surgery per standard of care. At the completion of the surgery, a second investigator (to reduce bias in data collection) will wear the cancer vision goggle (CVG) to visualize the excised tissue to determine if there are any positive margins based on the presence of LS301 fluorescence. If any positive margins are observed, the subject will be considered to have a positive margin; otherwise, the margin is considered negative. The excised tissue will later be examined by a breast cancer pathologist. Standard pathologic techniques will be used to determine if there are positive margins. Particular attention will be paid to the sites marked following CVG assessment to confirm whether the margin is positive or negative at any of these sites, or positive at other locations not identified by CVG. No interventions will be allowed based on the CVG results.

8.2 Endpoints

8.2.1 Endpoints for Safety Analysis

The primary endpoints of the phase 1 portion of the trial are toxicities and/or adverse events (as outlined in the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0) recorded after LS301 injection which are definitively or possibly related to the injection. Safety evaluation includes vital signs, clinical laboratory testing and ECG, measured pre- (within 10-15 minutes or 30 minutes of injection) and post-injection (within 30 minutes or/and at ~60 minutes). Noteworthy post-versus-pre changes in all the safety measures will be recorded while clinically significant changes will be reported as adverse events in accordance to HRPO requirements. All the safety endpoints are described in detail in Section 5.4 and adverse events detailed in Section 6.0.

8.2.2 Endpoint for Prediction of Positive Margin and Positive SLN Analysis

The primary objective is to assess margin status called by LS301 and CVG in comparison to the gold standard histopathological results. The histopathological results of margin positivity has the following possible results: margin negative, margin positive at LS301 and CVG identified locations, margin positive at LS301 and CVG unidentified locations. Using LS301 and CVG, the exercised tissue will be considered to be margin positive and have ink marked at the identified locations if any positive margins are observed; otherwise, the margin is considered negative. Diagnostic test characteristics (sensitivity and specificity) of LS301 and CVG for margin positivity by histology will established and margin positivity rate, defined as the proportion of tumors with histopathologically confirmed positive margins but missed by CVG, will be estimated. The secondary endpoint of the phase 2 trial is toxicity.

8.3 Data Analysis

Clinical and demographic characteristics of the participants will be summarized using descriptive statistics, overall and by dose levels.

8.3.1 Safety Analysis

Adverse events will be summarized by descriptive statistics (counts, percentages) overall and by dose level (if applicable). The post-injection vital signs will be separately compared to the pre-injection measurements by two-sample t-test or Wilcoxon rank sum test as appropriate, across all patients and by dose level (if applicable).

8.3.2 Margin Positivity and SLN Positivity Prediction Analysis

Margin positivity as determined by LS301 and CVG will be tabulated with the gold standard histopathological examination results as the table shown below.

		Histopathology results		
		Negative	Positive at CVG identified locations	Positive at other unidentified locations
CVG results	Negative	n_{11}	0	n_{13}
	Positive at CVG identified locations	n_{21}	n_{22}	0

Diagnostic test operating characteristics including sensitivity, specificity, overall accuracy, positive and negative predictive value will be calculated. The true positives (=n_{22}) are the tumors which show positive margins at any location agreed by both the histopathology examination and by LS301 and CVG. Specificity is defined as the proportion of tumors with histopathologically confirmed negative margins which are called to have negative margins by LS301 and CVG, calculated as $n_{11}/(n_{11}+ n_{21})$. Sensitivity is defined as the proportion of tumors which are predicted to have positive margins by LS301 and CVG among the tumors with histopathologically confirmed positive margins both at CVG identified locations and at other locations unidentified by CVG, calculated as $n_{22}/(n_{13}+n_{22})$. Overall accuracy is defined as the proportion of tumors with margin positivity/negativity correctly predicted by the CVG among all tumors, calculated as $(n_{11}+ n_{22})/(n_{11}+n_{13}+ n_{21}+n_{22})$. Positive predictive value is defined as the proportion of tumors with margins called positive at LS301 and CVG identified locations by the histopathology examination among all those predicted to have positive margins by CVG, calculated as $n_{22}/(n_{21}+n_{22})$. Negative predictive value was defined as the proportion of tumors with truly negative margins among those predicted so by CVG, calculated as $n_{11}/(n_{11}+ n_{13})$. The margin positivity rate based on LS301 and CVG will be estimated as $n_{13}/(n_{11}+n_{13}+ n_{21}+n_{22})$. All the estimates will be accompanied with 95% confidence interval. Margin positivity rate will be compared against the current 15.5% margin positivity rate using the two-sided one-sample binomial exact test. Given enough DCIS and invasive breast cancer (IBC), similar diagnostic characteristics will be calculated separately in the subset tumors of DCIS and IBC.

8.4 Sample Size

The sample size for the phase 1 part will depend on the observed DLT at each dose levels. The phase 1 expansion cohort will use 9 evaluable patients. The table below provides the probability of observing DLT in >=33% in 9 patients based on the binomial distribution. If the true DLT rate is as low as 5%, such a probability is small, only 0.0084 but if the true DTL is 50%, such a probability is very high at 0.91.

True DLT	probability of observed DLT >=33% in 9 patients
1%	0.000084
5%	0.0084
10%	0.053
20%	0.262
30%	0.537
40%	0.768
50%	0.91

The sample size for the phase 2 portion of the study was determined using the following calculations. A total of 88 (14 are expected to have positive margins after standard breast surgery) simultaneously achieves 84.7% power to detect an expected sensitivity of 0.95 against an unacceptable sensitivity of 0.7 and 100% power to detect an expected specificity 0.95 against an unfavorable specificity of 0.7 using a one-sided binomial test. The target significance level is 0.05. The actual significance level achieved by the test on sensitivity is 0.0475 and achieved by the test on specificity is 0.041. The current recall rate (i.e., margin positivity rate) after the use of cavity shave margin is ~19% [36], which agrees with the best practices in Washington University, ~15.5%. 88 patients allows 92.66% power to test a reduction in positive margin rate from 15.5% to 5% based on a one-sided Binomial exact test at a 5% level.

8.5 Early stopping rule

We don't expect any toxicities for LS301 but grade 1 toxicity is usually acceptable for imaging agent. As such, the trial will stop early in the phase 1 part following the rolling six design rule if dose level 1 has two out of six patients experience DLTs.

8.6 Analysis set

The safety analysis set includes all patients who receive any amount of LS301.

The efficacy analysis set include all patients who receive any amount of LS301, complete the surgery and have evaluable tumor histology results.

9.0 REFERENCES

1. Chen, S.L., Iddings, D.M., Scheri, R.P. & Bilchik, A.J. Lymphatic mapping and sentinel node analysis: current concepts and applications. *CA Cancer J. Clin.* **56**, 292-309 (2006).
2. Moran, M.S., *et al.* Society of Surgical Oncology–American Society for Radiation Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Stages I and II Invasive Breast Cancer. *Journal of Clinical Oncology* (2014).
3. Jacobs, L. Positive margins: The challenge continues for breast surgeons. *Ann. Surg. Oncol.* **15**, 1271-1272 (2008).
4. Collins, L., *et al.* Outcome of Women with Ductal Carcinoma In Situ (DCIS) Treated with Breast-Conserving Surgery Alone: A Case-Control Study of 225 Patients from the Cancer Research Network. *Mod. Pathol.* **22**, 34-35 (2009).
5. Vicini, F.A., *et al.* Impact of young age on outcome in patients with ductal carcinoma-in-situ treated with breast-conserving therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **18**, 296-306 (2000).
6. McCahill, L.E., *et al.* Variability in reexcision following breast conservation surgery. *Jama* **307**, 467-475 (2012).
7. Morrow, M., *et al.* Surgeon recommendations and receipt of mastectomy for treatment of breast cancer. *Jama* **302**, 1551-1556 (2009).
8. Mullen, R., *et al.* Involved anterior margins after breast conserving surgery: Is re-excision required? *European Journal of Surgical Oncology* **38**, 302-306.
9. Skripenova, S. & Layfield, L.J. Initial margin status for invasive ductal carcinoma of the breast and subsequent identification of carcinoma in reexcision specimens. *Archives of pathology & laboratory medicine* **134**, 109-114 (2010).
10. Jacobs, L. Positive margins: The challenge continues for breast surgeons. *Annals of Surgical Oncology* **15**, 1271-1272 (2008).
11. Zavagno, G., *et al.* Role of resection margins in patients treated with breast conservation surgery. *Cancer* **112**, 1923-1931 (2008).
12. Giuliano, A.E., Kirgan, D.M., Guenther, J.M. & Morton, D.L. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Annals of surgery* **220**, 391-398; discussion 398-401 (1994).
13. Cox, C.E., *et al.* Guidelines for sentinel node biopsy and lymphatic mapping of patients with breast cancer. *Annals of surgery* **227**, 645-651; discussion 651-643 (1998).
14. Nieweg, O.E. & Veenstra, H.J. False-negative sentinel node biopsy in melanoma. *J. Surg. Oncol.* **104**, 709-710 (2011).
15. Bezu, C., *et al.* Anaphylactic response to blue dye during sentinel lymph node biopsy. *Surg. Oncol.* **20**, 55-59 (2011).
16. van den Berg, N.S., *et al.* Multimodal Surgical Guidance during Sentinel Node Biopsy for Melanoma: Combined Gamma Tracing and Fluorescence Imaging of the Sentinel Node through Use of the Hybrid Tracer Indocyanine Green-Tc-Nanocolloid. *Radiology*, 140322 (2014).
17. Arii, S., *et al.* Surgical strategies for hepatocellular carcinoma with special reference to anatomical hepatic resection and intraoperative contrast-enhanced ultrasonography. *Oncology* **78 Suppl 1**, 125-130 (2010).

18. van Vledder, M.G., *et al.* The effect of steatosis on echogenicity of colorectal liver metastases on intraoperative ultrasonography. *Arch Surg* **145**, 661-667 (2010).
19. Ukimura, O., *et al.* Intraoperative ultrasonography in an era of minimally invasive urology. *International journal of urology : official journal of the Japanese Urological Association* **15**, 673-680 (2008).
20. Kane, R.A. Intraoperative ultrasonography: history, current state of the art, and future directions. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* **23**, 1407-1420 (2004).
21. Rudin, M. & Weissleder, R. Molecular imaging in drug discovery and development. *Nat. Rev. Drug Discov.* **2**, 123-131 (2003).
22. Wilke, L.G., *et al.* Rapid noninvasive optical imaging of tissue composition in breast tumor margins. *American Journal of Surgery* **198**, 566-574 (2009).
23. Troyan, S.L., *et al.* The FLARE((TM)) Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Breast Cancer Sentinel Lymph Node Mapping. *Annals of Surgical Oncology* **16**, 2943-2952 (2009).
24. Keller, M.D., Majumder, S.K. & Mahadevan-Lansen, A. Spatially offset Raman spectroscopy of layered soft tissues. *Optics Letters* **34**, 926-928 (2009).
25. Bydlon, T.M., *et al.* Rapid Optical Imaging of Breast Tumor Margins: Final Results from a 100-Patient Clinical Study. *Cancer Research* **69**, 770S-771S (2009).
26. Bhushan, K.R., *et al.* Detection of Breast Cancer Microcalcifications Using a Dual-modality SPECT/NIR Fluorescent Probe. *Journal of the American Chemical Society* **130**, 17648-+ (2008).
27. Marzullo, A.C.D., Neto, O.P., Bitar, R.A., Martinho, H.D. & Martin, A.A. FT-Raman spectra of the border of infiltrating ductal carcinoma lesions. *Photomedicine and Laser Surgery* **25**, 455-460 (2007).
28. Zysk, A.M. & Boppart, S.A. Computational methods for analysis of human breast tumor tissue in optical coherence tomography images. *Journal of Biomedical Optics* **11**, - (2006).
29. Haka, A.S., *et al.* In vivo margin assessment during partial mastectomy breast surgery using Raman spectroscopy. *Cancer Research* **66**, 3317-3322 (2006).
30. Lenkinski, R.E., Ahmed, M., Zaheer, A., Frangioni, J.V. & Goldberg, S.N. Near-infrared fluorescence imaging of microcalcification in an animal model of breast cancer. *Academic Radiology* **10**, 1159-1164 (2003).
31. Zuzak, K.J., Schaeberle, M.D., Gladwin, M.T., Cannon, R.O. & Levin, I.W. Noninvasive determination of spatially resolved and time-resolved tissue perfusion in humans during nitric oxide inhibition and inhalation by use of a visible-reflectance hyperspectral imaging technique. *Circulation* **104**, 2905-2910 (2001).
32. Troyan, S.L., *et al.* The FLARE Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Breast Cancer Sentinel Lymph Node Mapping. *Ann. Surg. Oncol.* **16**, 2943-2952 (2009).
33. Hirche, C., *et al.* An experimental study to evaluate the Fluobeam 800 imaging system for fluorescence-guided lymphatic imaging and sentinel node biopsy. *Surgical innovation* **20**, 516-523 (2013).
34. Tobis, S., *et al.* Near infrared fluorescence imaging after intravenous indocyanine green: initial clinical experience with open partial nephrectomy for renal cortical tumors. *Urology* **79**, 958-964 (2012).

35. Gotoh, K., *et al.* A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. *J. Surg. Oncol.* **100**, 75-79 (2009).
36. Liu, Y., *et al.* Intraoperative detection of liver tumors aided by a fluorescence goggle system and multimodal imaging. *The Analyst* **138**, 2254-2257 (2013).
37. Liu, Y., *et al.* Hands-free, wireless goggles for near-infrared fluorescence and real-time image-guided surgery. *Surgery* **149**, 689-698 (2011).
38. Liu, Y., *et al.* Near-infrared fluorescence goggle system with complementary metal-oxide-semiconductor imaging sensor and see-through display. *Journal of biomedical optics* **18**, 101303 (2013).
39. Liu, Y., Solomon, M. & Achilefu, S. Perspectives and potential applications of nanomedicine in breast and prostate cancer. *Medicinal research reviews* **33**, 3-32 (2013).
40. Liu, Y., *et al.* Complementary fluorescence-polarization microscopy using division-of-focal-plane polarization imaging sensor. *Journal of biomedical optics* **17**, 116001 (2012).
41. Liu, Y., *et al.* First in-human intraoperative imaging of HCC using the fluorescence goggle system and transarterial delivery of near-infrared fluorescent imaging agent: a pilot study. *Translational research : the journal of laboratory and clinical medicine* **162**, 324-331 (2013).
42. Mondal, S.B., *et al.* Real-time fluorescence image-guided oncologic surgery. *Advances in cancer research* **124**, 171-211 (2014).
43. Mondal, S.B., *et al.* Binocular Goggle Augmented Imaging and Navigation System provides real-time fluorescence image guidance for tumor resection and sentinel lymph node mapping. *Sci Rep* **5**, 12117 (2015).
44. Kramer-Marek, G., Gore, J. & Korc, M. Molecular imaging in pancreatic cancer--a roadmap for therapeutic decisions. *Cancer letters* **341**, 132-138 (2013).
45. Yang, L., Cao, Z., Lin, Y., Wood, W.C. & Staley, C.A. Molecular beacon imaging of tumor marker gene expression in pancreatic cancer cells. *Cancer biology & therapy* **4**, 561-570 (2005).
46. Bares, R., *et al.* F-18 fluorodeoxyglucose PET in vivo evaluation of pancreatic glucose metabolism for detection of pancreatic cancer. *Radiology* **192**, 79-86 (1994).
47. Trajkovic-Arsic, M., *et al.* Multimodal molecular imaging of integrin alphavbeta3 for in vivo detection of pancreatic cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **55**, 446-451 (2014).
48. Carlson, S.K., *et al.* Quantitative molecular imaging of viral therapy for pancreatic cancer using an engineered measles virus expressing the sodium-iodide symporter reporter gene. *AJR. American journal of roentgenology* **192**, 279-287 (2009).
49. Eser, S., *et al.* In vivo diagnosis of murine pancreatic intraepithelial neoplasia and early-stage pancreatic cancer by molecular imaging. *Proc Natl Acad Sci U S A* **108**, 9945-9950 (2011).
50. He, Y., *et al.* Anti-CXCR4 monoclonal antibody conjugated to ultrasmall superparamagnetic iron oxide nanoparticles in an application of MR molecular imaging of pancreatic cancer cell lines. *Acta radiologica* **53**, 1049-1058 (2012).
51. Yang, L., *et al.* Molecular imaging of pancreatic cancer in an animal model using targeted multifunctional nanoparticles. *Gastroenterology* **136**, 1514-1525 e1512 (2009).
52. Keliher, E.J., *et al.* Targeting cathepsin E in pancreatic cancer by a small molecule allows in vivo detection. *Neoplasia* **15**, 684-693 (2013).

53. Liu, Z., *et al.* Integrin alphavbeta6-Targeted SPECT Imaging for Pancreatic Cancer Detection. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **55**, 989-994 (2014).
54. Bremer, C., Tung, C.H. & Weissleder, R. Molecular imaging of MMP expression and therapeutic MMP inhibition. *Academic radiology* **9 Suppl 2**, S314-315 (2002).
55. Lee, H., *et al.* Near-infrared pH-activatable fluorescent probes for imaging primary and metastatic breast tumors. *Bioconjugate chemistry* **22**, 777-784 (2011).
56. Urano, Y., *et al.* Selective molecular imaging of viable cancer cells with pH-activatable fluorescence probes. *Nature medicine* **15**, 104-109 (2009).
57. Achilefu, S., Dorshow, R.B., Bugaj, J.E. & Rajagopalan, R. Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging. *Investigative radiology* **35**, 479-485 (2000).
58. Bugaj, J.E., Achilefu, S., Dorshow, R.B. & Rajagopalan, R. Novel fluorescent contrast agents for optical imaging of in vivo tumors based on a receptor-targeted dye-peptide conjugate platform. *Journal of biomedical optics* **6**, 122-133 (2001).
59. Achilefu, S., Wilhelm, R.R., Jimenez, H.N., Schmidt, M.A. & Srinivasan, A. A new method for the synthesis of tri-tert-butyl diethylenetriaminepentaacetic acid and its derivatives. *The Journal of organic chemistry* **65**, 1562-1565 (2000).
60. Achilefu, S., *et al.* Synergistic effects of light-emitting probes and peptides for targeting and monitoring integrin expression. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 7976-7981 (2005).
61. Achilefu, S. Lighting up tumors with receptor-specific optical molecular probes. *Technology in Cancer Research & Treatment* **3**, 393-409 (2004).
62. Achilefu, S., Dorshow, R.B., Bugaj, J.E. & Rajagopalan, R. Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging. *Investigative Radiology* **35**, 479-485 (2000).
63. Berezin, M.Y., *et al.* Rational approach to select small peptide molecular probes labeled with fluorescent cyanine dyes for in vivo optical imaging. *Biochemistry* **50**, 2691-2700 (2011).
64. Goiffon, R.J., Akers, W.J., Berezin, M.Y., Lee, H. & Achilefu, S. Dynamic noninvasive monitoring of renal function in vivo by fluorescence lifetime imaging. *Journal of biomedical optics* **14**, 020501 (2009).
65. Thota, R., Pauff, J.M. & Berlin, J.D. Treatment of metastatic pancreatic adenocarcinoma: a review. *Oncology* **28**, 70-74 (2014).
66. Al-Hajeili, M., Azmi, A.S. & Choi, M. Nab-paclitaxel: potential for the treatment of advanced pancreatic cancer. *OncoTargets and therapy* **7**, 187-192 (2014).
67. Kratz, F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *Journal of controlled release : official journal of the Controlled Release Society* **132**, 171-183 (2008).
68. Charanya, T., *et al.* Trimodal color-fluorescence-polarization endoscopy aided by a tumor selective molecular probe accurately detects flat lesions in colitis-associated cancer. *Journal of biomedical optics* **19**, 126002 (2014).
69. Rosenthal, E.L., *et al.* Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. *Clinical Cancer Research* **21**, 3658-3666 (2015).
70. Zinn, K.R., *et al.* IND-Directed Safety and Biodistribution Study of Intravenously Injected Cetuximab-IRDye800 in Cynomolgus Macaques. *Molecular imaging and*

biology : MIB : the official publication of the Academy of Molecular Imaging **17**, 49-57 (2015).
71. Gaykema, S.B.M., *et al.* 89Zr-Bevacizumab PET Imaging in Primary Breast Cancer. *Journal of Nuclear Medicine* **54**, 1014-1018 (2013).
72. Franklin, H.L., Miller, D.M., Hedges, T., Perry, J. & Parrish-Novak, J. Clinical development of BLZ-100 for real-time optical imaging of tumors during resection. Vol. 9696 96960V-96960V-96967 (2016).
73. Fidel, J., *et al.* Preclinical Validation of the Utility of BLZ-100 in Providing Fluorescence Contrast for Imaging Spontaneous Solid Tumors. *Cancer Research* **75**, 4283-4291 (2015).
74. Skolnik JM, Barrett JS, Jayaraman B, Patel D, Adamson PC (2008) Shortening the timeline of pediatric phase I trials: the rolling six design. *J Clin Oncol* **26**, 190-195.
75. Doussau A, Geoerger B, Jimenez I, Paoletti X. Innovations for phase I dose-finding designs in pediatric oncology clinical trials. Contemporary clinical trials. 2016. 47: 217-227

10.0 APPENDIX A: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

11.0 APPENDIX B: Reporting Timelines

	Expedited Reporting Timelines				
Event	**HRPO – Washington University**	**HRPPO – UT Southwestern**	**QASMC – Washington University**	**DSMC – UT Southwestern**	**FDA**
Serious AND unexpected suspected adverse reaction				Submit SAE Report to SCCC DSMC within 5 business days of study team awareness of the event(s)	Report no later than 15 calendar days after it is determined that the information qualifies for reporting
Unexpected fatal or life-threatening suspected adverse reaction				Submit within 5 business days of study team awareness of the event(s)	Report no later than 7 calendar days after initial receipt of the information
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Submit within 5 business days of study team awareness of the event(s)	Report via email after IRB acknowledgment	Submit UPIRSO Report to SCCC DSMC within 5 business days of study team awareness of the event(s)	
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Submit within 5 business days of study team awareness of the event(s)			Report no later than 15 calendar days after it is determined that the information qualifies for reporting

	Expedited Reporting Timelines				
Event	**HRPO – Washington University**	**HRPPO – UT Southwestern**	**QASMC – Washington University**	**DSMC – UT Southwestern**	**FDA**
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.	Submit within 5 business days of study team awareness of the event(s)			
Protocol exception	Approval must be obtained prior to implementing the change	Approval must be obtained prior to implementing the change			
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB					Report no later than 15 calendar days after it is determined that the information qualifies for reporting
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.	Submit within 5 business days of study team awareness of the event(s)			
Breach of confidentiality	Within 10 working days.				

Expedited Reporting Timelines					
Event	**HRPO – Washington University**	**HRPPO – UT Southwestern**	**QASMC – Washington University**	**DSMC – UT Southwestern**	**FDA**
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.				

Routine Reporting Timelines					
Event	**HRPO – Washington University**	**HRPPO – UT Southwestern**	**QASMC – Washington University**	**DSMC – UT Southwestern**	**FDA**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported at Continuing Review.	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	Adverse events will be reviewed at Annual DSMC Review	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.
Minor deviation	Report summary information at the time of continuing review.	Minor deviations will be reported at Continuing Review.		Minor deviations will be reviewed at Annual DSMC Review	

Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.	All complaints are summarized in the next progress report submitted as part of continuation review or in the final report submitted to inactivate the study			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.				

12.0 APPENDIX C: Washington University Unanticipated Problem Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Admission Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**

13.0 APPENDIX D: Washington University Unanticipated Problem Reporting Cover Sheet

UT Southwestern Harold C. Simmons Comprehensive Cancer Center | NCI Designated Comprehensive Cancer Center

DSMC Serious Adverse Event Report Form

Protocol Name:									
IRB #:				PI:					
Subject Initials				Study ID:					
☐ Initial Report	☐ Follow Up #_____								
Serious Adverse Event / **Grade**:									
Onset Date:				Resolution Date:					
Date Study Team became aware of the event(s):									
Relationship to study drug:	☐ Not Related ☐ Unlikely Related ☐ Possibly Related ☐ Probably Related ☐ Definitely Related								
Is the event unexpected in terms of nature, severity or frequency?	☐ Yes ☐ No								
Do any of the events place the subject or others at greater risk of harm?	☐ Yes ☐ No If yes explain:								
Study Drug Name or Type of Radiation Therapy:	Date of prior dose before the event:								
Dose level:	Cohort Number:								
Brief Description of the Event:									
Action with Study Drug or RT:	☐ Dose not changed ☐ Dose reduction ☐ Temporarily delayed ☐ Discontinued Permanently			Outcome:	☐ Recovered ☐ Recovered with sequelae ☐ Recovering ☐ Not Recovered ☐ Unknown				

Reporter Name: Please Print	Date:
Investigator's Name: Please Print	
Investigator Signature:	Date: / /
Investigator's Name: _____ Please Print	For Multi-Center Studies Only
Investigator Signature:	Date: / /

Scan and email the completed report with source documentation (if available) to: SCCDSMC@UTSouthwestern.edu for reporting to the DSMC (Data Safety and Monitoring Committee)