OncoNano Medicine Inc.
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Protocol and Protocol Amendments

Title	A Phase 2, Single-Dose, Open-Label Study to Evaluate Diagnostic Performance and Safety of Pegsitacianine, An Intraoperative Fluorescence Imaging Agent for the Detection of Lung Malignancies, In Patients Undergoing Routine Surgery
Study Drug	Pegsitacianine (ONM-100)
Protocol Amendment 2.0	25 January 2022
Original Protocol	31 August 2021

STUDY PROTOCOL

A PHASE 2, SINGLE-DOSE, OPEN-LABEL STUDY TO EVALUATE DIAGNOSTIC PERFORMANCE AND SAFETY OF PEGSITACIANINE, AN INTRAOPERATIVE FLUORESCENCE IMAGING AGENT FOR THE DETECTION OF LUNG MALIGNANCIES, IN PATIENTS UNDERGOING ROUTINE SURGERY

PROTOCOL NUMBER: ON-1005

Name of Drug:	Pegsitacianine (ONM-100)
Phase of Development:	Phase 2
Sponsor:	OncoNano Medicine
Sponsor Contact:	
Project Manager:	
Medical Monitor:	
Version:	2
Date of Protocol:	25 Jan 2022
Proprietary Notice:	The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of OncoNano.
Compliance Statement:	The study will be completed according to the guidelines of International Conference on Harmonisation Good Clinical Practice. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

SIGNATURE PAGE

PROTOCOL TITLE: A Phase 2, single-dose, open-label study to evaluate diagnostic performance and safety of pegsitacianine, an intraoperative fluorescence imaging agent for the detection of lung malignancies, in patients undergoing routine surgery

PROTOCOL NUMBER: ON-1005

DocuSigned by:

D78E36A1EA57432ABDC0C113607DD948 1/25/2022

VP, Head of Clinical Operations Date

INVESTIGATOR STATEMENT

I agree to conduct the study as outlined in the protocol in accordance with accepted Good Clinical Practice, the guidelines and all applicable government regulations including 21 CFR 54.

I have read and understand all sections of the protocol.

<Principal Investigator's Name> Date

REVISION HISTORY

Version Number	Date	Summary of Changes
1	31 Aug 2021	Original
2	25 Jan 2022	• Updated the Medical Monitor • Removal of lab values from exclusion criteria • Updated Inclusion Criteria for clarification • Updated safety information for completed 1002 study • Removed hypothesis testing from sample size calculation • Included description of site personnel training and responsibilities • Updated Requirements for Part 2 enrollment • Updated Statistical analysis to account for correlation • Clarification of Pegsitacianine as a novel drug • Updated Intent to treat, safety, efficacy populations • Removal of the any additional investigational drug restriction 30 days prior • Updated definitions of parameters of Imaging Analysis

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PROTOCOL SUMMARY

Protocol Number	ON-1005
Title of Trial	A Phase 2, single-dose, open-label study to evaluate diagnostic performance and safety of pegsitacianine, an intraoperative fluorescence imaging agent, for the detection of lung malignancies in patients undergoing routine surgery
Phase of Clinical Trial	Phase 2
Indications	Pegsitacianine is indicated as an imaging agent for intraoperative detection of lung malignancies in the thoracic cavity during routine surgical resection. Pegsitacianine is administered as a single dose prior to surgery.
Study Centers	up to 5 centers
Trial Objectives	Primary Objective: The primary objective of this study is to determine if administration of pegsitacianine (mg/kg) results in a clinically significant event at the level of the patient. Examples of clinically significant events include: 1. The detection of disease that cannot be detected using standard procedures 2. The detection of synchronous lesions 3. The detection of positive surgical margins following primary tumor resection 4. Tumor-negative standard of care (SOC) biopsy Secondary Objectives: Key secondary objectives are to evaluate the safety and toxicity of pegsitacianine. Additionally, the sensitivity, specificity, negative predictive values, and positive predictive values of the imaging agent will be calculated at the level of the individual specimens, including lymph nodes.
Background	Pegsitacianine is a novel intraoperative nanoparticle-based fluorescence imaging agent that was evaluated during the first-in-human Phase 1 study (ON-1001). ON-1001 was completed in the Netherlands in patients with solid cancers undergoing surgical excision of their tumors. An additional Phase 1 study (ON-1004) evaluating the pharmacokinetics in a healthy volunteer population has also been completed. Pegsitacianine was further evaluated in a completed Phase 2 study (ON-1002) in solid tumors. Pegsitacianine fluorescence is quenched when exposed to normal physiological pH but becomes activated following micellar disassembly in the acidic tumor microenvironment. Pegsitacianine was demonstrated to be well tolerated and showed no dose-limiting toxicities (DLT).
Trial Design	This Phase 2 study will be an interventional, open-label, single arm trial where each patient is his/her own "intrapatient" control. All patients will receive a single mg/kg dose of pegsitacianine prior to standard of care (SOC) surgery. It is recommended by the sponsor to administer prophylactic diphenhydramine before administration of pegsitacianine to decrease the possibility of an infusion-related reaction. The study will be conducted in two Parts. Part 1 will enroll patients undergoing minimally invasive procedures (i.e., video-assisted thorascopic surgery [VATS] or robotic surgery). Part 2 will be opened at the discretion of the Sponsor and include patients undergoing open thoracotomy for the removal of their disease. Enrollment will be open first to Part 1 with the opportunity to open Part 2 for enrollment following the demonstration of

	satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Part 1. Patients will have a biopsy confirmed diagnosis, or a high clinical suspicion of a lung malignancy based on MRI, CT and/or PET imaging. A total of up to 40 patients will be enrolled in this trial across both parts. The sponsor reserves the right to perform an interim analysis following early enrollment ($n \leq 10$ patients) to determine the feasibility and benefit of pegsitacianine as an adjunct to SOC surgery. <u>Parts 1 and 2:</u> The surgeon will attempt to perform their SOC resection of visible tumors. If the tumor is unable to be located using standard surgical approaches, an appropriate near-infrared (NIR) imaging device may be used to aid in localizing the primary tumor. Localization of the primary tumor using NIR imaging in conjunction with pathology confirmed presence of disease will be considered a clinically significant event. If the primary tumor is located using standard operative procedures, the tumor will be imaged using the NIR camera and removed as per standard of care (i.e., wedge, lobectomy, etc.). The primary resection will then be imaged (front, back and margins) on a piece of normal tissue within the surgical field at a distance from the specimen equal to the working distance of the camera system. Inspection and imaging of the resection margins will be performed using the NIR camera system. If an area of suspicion is observed, images will be captured of the area, at the working distance of the camera away from the margin and documented for future correlation to pathological findings. Similarly, the *in situ* resection margin on the lung will be imaged for signs of residual fluorescence that may correspond to disease left behind. If found, images will be captured prior to the excision of the margin (*in situ*) and following its resection on a piece of normal tissue (*ex situ*). Resection of the suspicious or fluorescent margin is at the discretion of the surgeon. This procedure is to be repeated if additional SOC nodules are planned for resection. An identical approach will be followed for any SOC lymph nodes collected during the procedure. Lymph node imaging will occur prior to and following the removal of the suspected lymph nodes. Once SOC surgical resection is complete, the surgeon will use the NIR camera system to evaluate the thoracic cavity (i.e., chest wall, contralateral lung, bronchi, etc.) for indications of residual fluorescence. If additional fluorescent foci are observed, the area may be resected, at the discretion of the surgeon, for pathological confirmation of disease. Similar to the imaging of margin resections, the suspected area will be imaged prior to removal (*in situ*) and following resection (*ex situ*) on a piece of normal tissue within the thoracic cavity. Additional fluorescent lymph nodes, detected during the evaluation of the thoracic cavity, may also be collected and imaged both *in situ* and *ex situ* prior to being evaluated in pathology. Collected surgical specimens will be documented by the clinical research coordinator, as dictated by the surgeon in the provided Imaging Workbook. The fluorescence status of each specimen will be assessed and called out by the surgeon (fluorescent or not fluorescent), and similarly documented in the workbook. All samples will be sent to pathology for routine analysis. To determine the performance

	metrics of pegsitacianine, the fluorescence status of each specimen will be correlated to the final pathology and results will be tabulated.
Endpoints	Primary Endpoints: Discovery of a clinically significant event (CSE) at the level of the subject. CSEs may include detection of primary tumors that went undetected under white light, discovery of occult disease, positive margins detected by fluorescence that would have otherwise gone undetected, or tumor-negative SOC biopsies. Secondary Endpoints: Secondary endpoints will evaluate the safety and toxicity of pegsitacianine, as well as performance metrics of the drug at the level of the collected specimens. The performance metrics will include: • Sensitivity • Specificity • NPV • PPV
Study Drug	mg/kg of pegsitacianine prior to surgery
Eligibility	Inclusion Criteria: 1. Adults 18 years of age and older 2. Biopsy confirmed diagnosis, or a high clinical suspicion of a lung malignancy based on MRI, CT and/or PET imaging. 3. Candidates for surgery as determined by the Investigator. 4. Documented negative serum or urine pregnancy test for women of childbearing potential. 5. Male patients and female patients of child-bearing potential (i.e. premenopausal women with intact reproductive organs and women <2 years after menopause) must agree to, and comply with using medically acceptable contraception including surgical sterilization (e.g. hysterectomy, bilateral oophorectomy, bilateral tubal ligation), intrauterine device, oral contraceptive, contraceptive patch, long acting injectable contraceptive, partner's vasectomy, double-barrier method (condom or diaphragm plus spermicide or condom plus diaphragm), or abstinence during the trial and for 6 months thereafter 6. Agreement to abstain from alcohol consumption 72 hours prior to drug administration and for minimum of 10 days post-surgery 7. Agreement to complete all follow-up visits 8. Willing and able to provide written informed consent Exclusion Criteria: 1. Known hypersensitivity or allergy to indocyanine green (ICG), p[redacted] polyethylene glycol (PEG). 2. Tumor locations the surgeon deems unfeasible to image intraoperatively 3. Excessive and/or generalized disease deemed inoperable by the surgeon 4. Life expectancy less than 12 weeks 5. Karnofsky Performance Status less than 70% 6. Hepatic impairment that in the opinion of the investigator would exclude the subject from the study 7. Lab values that in the opinion of the investigator would prevent surgery

	8. Pregnant or lactating 9. Taking or plan to take medications with known hepatoxicity 10. Any other significant medical condition the investigator deems inappropriate for the trial including any medical or psychiatric conditions that would impair informed consent
Sample Size	N=40 Up to 40 patients will be enrolled in this study. No set allocation of patients to Part 1 and Part 2 will be implemented, rather Part 2 will be opened at the discretion of the sponsor following the demonstration of satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Part 1. The sponsor reserves the right to perform an interim analysis following early enrollment (i.e., $n \leq 10$ patients) to determine the feasibility and benefit of pegsitacianine as an adjunct to standard of care lung malignancy resection.
Statistical Analysis	The quotient of patients with a CSE over the total number of patients undergoing surgery who received any dose of the study drug, and had sufficient imaging data (minimum of 1 collected image). Sensitivity and specificity will be determined at the specimen level based on correlated fluorescence status and pathological outcome. Pegsitacianine performance data will be evaluated *in toto*, as well as separately if Part 2 of the study is opened to enrollment. Part 1 and Part 2 data will be evaluated independently to assess performance when using different imaging systems within their respective procedure (i.e., minimally invasive vs. open).
Schedule of Events	**Screening (Day -30 to -1)** Before the study doctor can administer pegsitacianine, he/she must ensure that it is safe for the patient to be in the study vis-à-vis information that is already known about pegsitacianine. After the patient has agreed to be in the study and signed the informed consent form, the study team will perform the following procedures (information collected as part of standard of care within a reasonable timeframe that meet the presurgical requirements of the institution are also acceptable): • Collect demographic data • Record their complete medical history • Perform a complete physical examination • Measure their weight and height and calculate a body mass index (BMI) to estimate body fat tissue • Record vital signs (temperature, heart rate, blood pressure, and respirations) • Obtain a blood sample for routine laboratory tests, including a pregnancy test for females who can become pregnant • Record medications they have been taking, including prescription, over-the-counter, and herbal medications • Determine Karnofsky Performance Status (to assess the ability to perform ordinary daily tasks) • Confirm that the patient has met all the study criteria to participate **Day 0 (Pegsitacianine administration)** **Predose procedures**

- Confirm eligibility for the study and that medical information is complete
- Record weight
- Discuss how the patient is feeling and their everyday activities
- Record vital signs
- Perform a 12-lead electrocardiogram (ECG) to check the heart activity
- Install an IV line
- Obtain blood samples for routine laboratory tests, including a pregnancy test for females who can become pregnant
- Record all medications taken since the Screening visit, including prescription, over-the-counter, and herbal medications to confirm no prohibited medications have been taken

The Sponsor recommends the use of prophylactic diphenhydramine prior to study drug administration, to decrease the possibility of an infusion-related reaction.

Dosing and postdose procedures

Procedures during and after dosing of pegsitacianine are summarized below:

- Dosing of pegsitacianine will be administered
  The total dosing time will be dependent on the patient's body weight and total volume to be infused.
- The following postdose assessments and procedures will occur after infusion of the study drug has been completed:
  - Record the patient's vital signs immediately after the infusion and 30 minutes afterward
  - Discuss any new or unexpected changes in how the patient is feeling
  - Record any AEs
  - Record all medications they are taking, including prescription, over-the-counter, and herbal medications

Surgery ([redacted]**)**

Procedures on the day of surgery

The following procedures will be performed on the day of surgery (before administration of anesthesia and before surgery):

- Record the patient's vital signs
- Record all medications being taken, including prescription, over-the-counter, and herbal medications
- Discuss with the patient any new or unexpected changes in how they are feeling
- Record any AEs
- Obtain a blood sample for routine laboratory tests

	Once the patient is under anesthesia, some procedures will occur that would not normally be part of surgery if the patient were not in this study. The SOC surgical procedures will be allowed to continue with the addition of the steps listed below. • If the primary tumor is not visually apparent using standard approaches, the NIR camera may be used to help locate and guide the resection of the primary tumor • If the primary tumor is apparent under standard conditions, the suspected area will be imaged using the intraoperative camera prior to excision, and on a section of normal tissue at a distance equal to the working distance of the camera following excision • Following the primary tumor resection, margins (both on the excised primary and the remaining lung) will be examined using NIR in an effort to detect the presence of a possible positive margin • Any SOC lymph nodes that are to be collected will be imaged prior to, and following excision in a manner identical to that performed on the primary tumor specimen • Once the SOC primary tumor and lymph node resection is complete, the surgeon will examine the thoracic cavity with the intraoperative NIR camera in search of additional fluorescent disease that was missed during SOC, or additional fluorescent lymph nodes • Additional fluorescent lesions will be imaged prior to excision, removed, and then imaged on a section of normal tissue in a manner analogous to the SOC specimens. **Day of Discharge** • Record the patient's vital signs • Perform a physical examination • Discuss new or unexpected changes in they are feeling • Obtain blood samples for routine laboratory tests • Record all medications being taken, including prescription, over-the-counter, and herbal medications • Discuss any new or unexpected changes • Record any AEs **Day 21 (±10 days) after dosing** • Record the patient's vital signs • Perform 12-lead ECG to monitor heart activity • Perform a physical examination • Discuss new or unexpected changes in how they are feeling • Obtain blood samples for routine laboratory tests, including a pregnancy test for females who can become pregnant • Record all medications being taken, including prescription, over-the-counter, and herbal medications • Discuss any new or unexpected changes • Record any AEs
Training	Extensive training will be provided to all study staff. Initial training will occur at the Site Initiation Visit, followed by on-site training that will take place during the initial surgical procedures with Sponsor Representatives present in the operating room during the procedures. Sponsor Representatives will ensure all study personnel

	are adequately trained and follow identical procedures to preserve the integrity of the collected data without providing input on the selection of specimens or their fluorescent status, these tasks are are the sole responsibility of the operating surgeon. Additional retraining may take place if necessary throughout the course of the study. Training will include a review of protocol procedures, NIR camera operating instructions, documentation of specimens and their fluorescence status, and upload of source documentation. All training will be documented and stored within the Trial Master File.

ABBREVIATIONS

ABBREVIATION	TERM
AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CSE	Clinically Significant Event
CT	Computed Tomography
CV	Curriculum Vitae
D5W	5% Dextrose in water
DoH	Declaration of Helsinki
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	United States Food and Drug Administration
HNSCC	Head and Neck Squamous Cell Carcinoma
ICG	Indocyanine Green
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
IV	Intravenous
MFI	Mean Fluorescence Intensity
MRI	Magnetic Resonance Imaging
NIR	Near-infrared
NPV	Negative Predictive Value
PEG	Polyethylene Glycol
PET	Positron Emission Tomography
PK	Pharmacokinetics
PMMA	Polymethylmethacrylate
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
SAE	Serious Adverse Event
SBR	Specimen-to-Background Ratio
SOC	Standard of Care
SUSAR	Suspected Unexpected Serious Adverse Reaction
SWI	Sterile Water for Injection
TBR	Tumor-to-Background Ratio

ABBREVIATION	**TERM**
TEAE	Treatment-Emergent Adverse Event
VATS	Video-Assisted Thorascopic Surgery
WMA	World Medical Association

1. INTRODUCTION

1.1 Overview

According to the World Health Organization, lung cancer is the most common cause of cancer-related death in men and women, and is responsible for 1.5 million deaths worldwide annually as of 2012.[1] Surgery remains the best option for patients presenting with operable Stage I or II cancers, however the five year survival rate, even after surgery, for these candidates remains at a dismal 73% for Stage I and 53% for Stage II.[2] At least a third of these patients recur locally.[3] Thus, the high rate of local recurrence suggest that surgeons are unable to completely detect and remove primary tumor nodules in a satisfactory manner as well as lingering metastases in sentinel lymph nodes. By ensuring a negative margin through the use of image-guided surgery it would be possible for to improve upon the rates of recurrence free patients and thus lead to improved overall survival.

During cancer surgery, surgeons are classically limited to two intraoperative tools, visual inspection under standard white light and finger palpation, to decide and execute critical choices which determine the outcome of a tumor resection. The fact still remains that the hands and eyes can only provide limited data and feedback at the time of surgery. Our group and others have hypothesized that fluorescent labeling of malignant cells during surgery will improve the intraoperative detection of cancer. Simply, if tumor cells are made to fluoresce or "glow" during surgery, the surgeons are more likely to identify tumor margins, residual disease, positive lymph nodes and satellite metastases based on the additional information provided by intraoperative NIR imaging.

This concept of intraoperative molecular imaging requires two new innovations: 1) a fluorescent imaging agent that can be injected systemically into the patient that selectively accumulates in the tumor tissues, and 2) a camera system that can detect and quantify the imaging agent in the tumor tissues. Our group has developed a new fluorescent imaging agent, pegsitacianine, to address these needs.

Pe sitacianine is a novel ultra-pH sensitive polymeric micelle consistin of a P [redacted] Covalent attachment of ICG to the [redacted] portion of the polymer results in [redacted]. Upon micelle localization to the acidic tumor microenvironment, e sitacianine disassembles into individual polymers ([redacted]) and the [redacted] Upon excitation of ICG with a suitable light source, the individual polymer is capable of fluorescing areas of disease. By targeting the universal biomarker of acidic pH in the tumor microenvironment, pegsitacianine is an ideal imaging agent for applications across a broad range of tumor indications.

1.2 Pegsitacianine Clinical Trial Background

Four clinical studies have been performed or are currently enrolling using pegsitacianine. The study *ON-1001: Image-Guided Surgery for Tumor Detection in Solid Tumors Using the pH Activated Micellar Probe ONM-100: The SHINE Study*, was conducted in the Netherlands at the

University Medical Center Groningen and completed in 2019.[7] The completed Phase 2 study, *ON-1002: A Study to Evaluate ONM-100, an Intraoperative Fluorescent Imaging Agent for the Detection of Cancer* (NCT03735680) was a multicenter trial in the United States. *ON-1004* was a Phase 1 normal healthy volunteer study to further evaluate the pharmacokinetic profile of pegsitacianine. *ON-1003: A Phase 2, Single-Dose, Open-Label Study to Evaluate Diagnostic Performance and Safety of Pegsitacianine, an Intraoperative Fluorescence Imaging Agent for the Detection of Peritoneal Metastases, in Patients Undergoing Cytoreductive Surgery* (NCT04950166) is an ongoing multicenter study in the United States.

The purpose of the ON-1001 study was to investigate the safety, pharmacokinetics and feasibility of pegsitacianine as an intraoperative imaging agent for the detection of tumors and metastatic lymph nodes in solid cancers. Additionally, the study investigated the optimal dose range of pegsitacianine for an adequate tumor-to-background/contrast-to-noise ratio of fluorescence obtained intraoperatively and with *ex vivo* specimens using indocyanine green (ICG) compatible cameras and imaging devices. A single pegsitacianine dose was administered [redacted] as a one to five-minute infusion to patients in five dose cohorts [redacted] with three patients per cohort in Phase 1A, and 15 patients at a dose of [redacted] in Phase 1B. Near-infrared (NIR) imaging was conducted intraoperatively, on the back table, and postoperatively of the primary tumor, lymph nodes, and pegsitacianine guided biopsies. Additionally, the bread loaf slices from the primary tumor were imaged postoperatively. The median [redacted] value from all [redacted] with an interquartile range of 3.1. Additionally, pegsitacianine demonstrated a sensitivity of 100% across all tumor t es tested with a s ecificit of 75% and [redacted]

The purpose of the ON-1002 study was to investigate whether pegsitacianine can be used to image primary tumors and metastatic lymph nodes using an imaging schedule earlier than [redacted] hours postdose in patients undergoing routine surgery of their solid cancers and whether the diagnostic performance to detect metastatic lymph nodes can be improved by optimizing the dose and the imaging schedule. The study was designed to be executed in three parts:

Part 1 was designed to evaluate the dose(s) at which pegsitacianine fluorescence imaging is feasible at 3 ±2 hours postdose and, if needed, at an alternate postdose imaging schedule, for the detection of metastatic lymph nodes and primary tumors after a single IV dose of pegsitacianine in patients with HNSCC or breast cancer undergoing routine surgery. Part 1 also evaluated safety at the dose(s) used to assess imaging feasibility and to select the dose(s) and postdose imaging schedule(s) that are safe and provide optimal imaging of solid tumors and metastatic lymph nodes; the dose and time postdose chosen for the detection of primary tumors and metastatic lymph nodes may be the same or different.

Part 2 of the ON-1002 study was designed to verify the safety and diagnostic performance of pegsitacianine compared to standard pathology at the dose(s) and postdose imaging schedule(s)

selected from Part 1 for the detection of the primary tumors and the metastatic lymph nodes in a variety of solid cancers (which may include HNSCC, breast cancer, colorectal cancer, urothelial cancer, prostate cancer, ovarian cancer and/or non-small-cell lung carcinoma [NSCLC]). In addition, the PK profile of pegsitacianine at the dose(s) and postdose imaging schedule(s) used to assess optimal imaging in Part 1 and Part 2 was assessed.

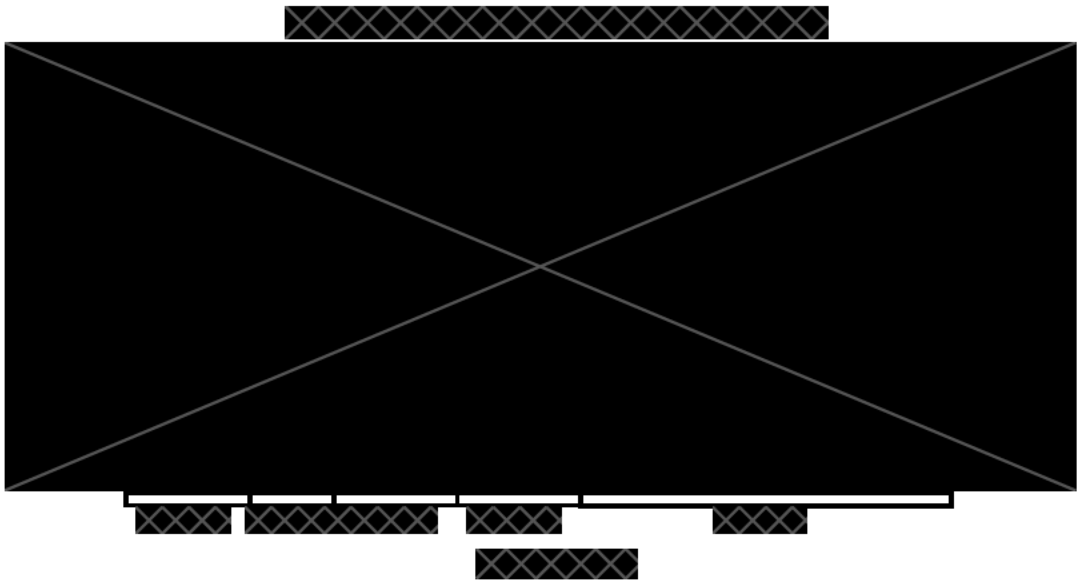
Part 3 was used to assess the safety and efficacy (sensitivity and positive predictive value [PPV]) of pegsitacianine at a dose of mg/kg for intraoperative imaging during HNSCC surgery, administered at [redacted]ours prior to surgery, in addition to a set of secondary and exploratory endpoints to further assess performance.

ON-1003 is an ongoing Phase 2 study designed to evaluate the ability of pegsitacianine to detect disease left behind following SOC surgical treatment of peritoneal carcinomatosis.

ON-1004 was a completed Phase 1 study that was conducted in normal, healthy volunteers. The study was designed to comprehensively evaluate the pharmacokinetic profile of pegsitacianine.

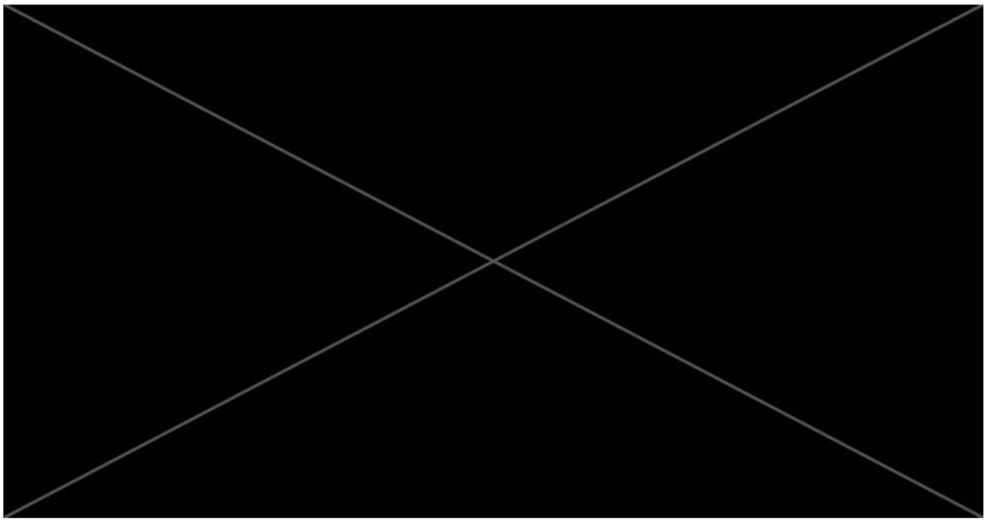
1.3 Pharmacodynamics and Dose Selection

The pharmacodynamic profile, and its relation to dose schedule selection of pegsitacianine was evaluated *in vivo* and *ex vivo* using intraoperative near-infrared cameras in both the ON-1001 and ON-1002 studies. The mean fluorescence intensities and [redacted] ratios for patients receiving [redacted] were not significantly different across the two studies to date. (The red line represents the [redacted] across all patients in each study).



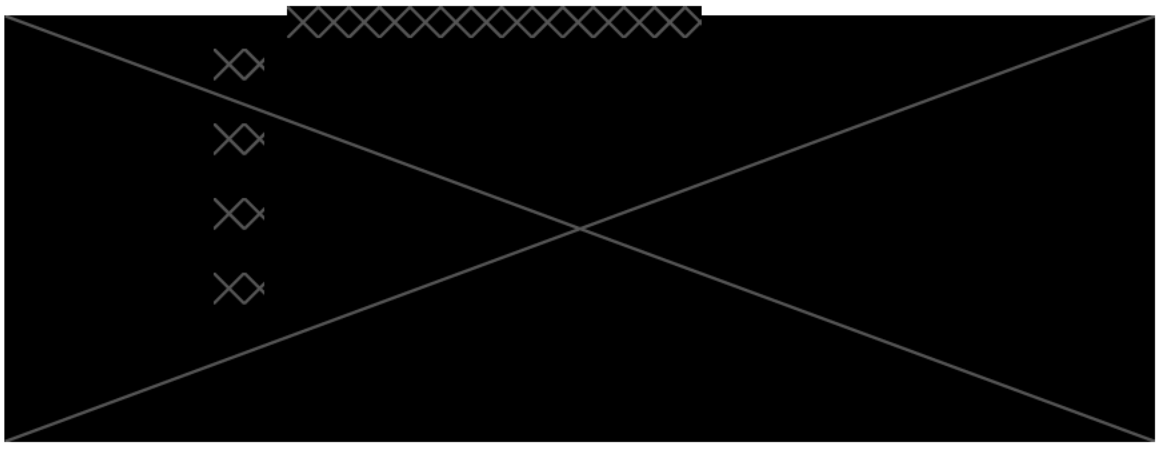
All dose levels investigated in the ON-1001 Phase 1 Trial were imaged [redacted] following the completion of the pegsitacianine infusion

Phase 2: In Vivo Tumor-to-Background Ratios



The ON-1002 Phase 2 Trial investigated a range of dose schedules across the patient population as depicted in the above figure

Calculated TBR ratios across all evaluable patients from both clinical studies show the absence of a dose schedule-to-TBR relationship. However, evaluation of mean fluorescence intensity (MFI) values of the tumor areas from the ON-1001 study do demonstrate a dose-dependent increases in MFI.



MFI values of tumor and normal tissue present on bread loaf slices as determined by pathology

Given the enhanced fluorescence of tumor areas at the higher doses of pegsitacianine, a dose of [redacted] chosen as the ideal dose for the continued evaluation of pegsitacianine in the completed ON-1002 study, as well as this ON-1005 clinical trial.

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to determine if administration of pegsitacianine (mg/kg) results in the detection of clinically significant events at the patient level.

Examples of clinically significant events include:

1. The detection of disease that cannot be detected using standard procedures during the surgical resection of lung malignancies
2. The detection of synchronous lesions
3. The detection of positive surgical margins following primary tumor resection
4. Tumor-negative SOC biopsies

2.2 Secondary Objectives

Key secondary objectives are to evaluate the safety and toxicity of pegsitacianine. Additionally, the sensitivity, specificity, negative predictive values, and positive predictive values of the imaging agent will be calculated at the level of the individual specimens. Combined and independent analysis of Part 1 and Part 2 performance metrics will be tabulated.

2.3 Exploratory Objectives

Assess the how the [redacted] affects the diagnostic accuracy of pegsitacianine in detection of metastatic disease vs. normal tissue.

3.STUDY PLAN

3.1 Overall Design

This Phase 2 study will be an interventional, open-label, single arm trial where each patient is his/her own "intrapatient" control. All patients will receive a single mg/kg dose of pegsitacianine prior to standard of care surgery.

Patients will have a biopsy confirmed diagnosis, or a high clinical suspicion of a lung malignancy based on MRI, CT and/or PET imaging. A total of up to 40 patients will be enrolled in this trial. Enrollment will be open first to Part 1 with the opportunity to open Part 2 for enrollment following the demonstration of satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Part 1. The sponsor reserves the right to perform an interim analysis following early enrollment (i.e., $n \leq 10$ patients) to determine the feasibility and benefit of pegsitacianine as an adjunct to standard of care surgical resection of lung malignancies. Interim analysis will assess the utility of pegsitacianine based on mean fluorescence intensity analysis, calculation of tumor to background ratios, calculation of sensitivity and specificity, safety, and investigator feedback.

The surgeon will begin by attempting to perform their SOC tumor resection. If the tumor is unable to be located under standard white light conditions, an appropriate FDA-cleared near-infrared imaging device (Examples found in **Appendix 2: FDA-Cleared NIR Imaging Devices**) may be used to aid in the location of the primary tumor. Localization of the primary tumor, when unable to be located using white light imaging, in conjunction with pathology confirmed presence of disease will be considered a clinically significant event.

If the primary tumor is located using standard white light procedures, the tumor will be imaged using the NIR camera and removed as per standard of care (i.e., wedge, lobectomy, etc.). The primary resection will then be imaged (front and back) on a piece of normal tissue within the surgical field at a distance from the specimen equal to the working distance of the camera system. Inspection and imaging of the resection margins will be performed using the NIR camera system. If an area of suspicion is observed, images will be captured of the area at the working distance of the camera away from the margin and documented for future correlation to pathological outcomes. Similarly, the resection margin on the lung will be imaged for signs or residual fluorescence that corresponds to residual disease left behind. If found, images will be captured prior to the excision of the margin (*in situ*) and following its resection on a piece of normal tissue (*ex situ*). This procedure is also to be repeated if additional SOC nodules are planned for resection. An identical approach will be followed for the collection of any SOC lymph nodes collected during the procedure. Lymph node imaging will occur prior to and following the removal of the suspected lymph nodes.

Once SOC surgical resection is complete, the surgeon will use the NIR camera system to evaluate the thoracic cavity (i.e., chest wall, contralateral lung, bronchi, etc.) for indications of residual fluorescence. If additional fluorescent foci are observed, the area may be resected at the discretion of the surgeon for pathological confirmation of disease. Similar to the imaging of margin resections, the suspected area will be imaged prior to removal (*in situ*) and following resection (*ex situ*) on a piece of normal tissue within the thoracic cavity. Additional fluorescent lymph nodes, detected during the evaluation of the thoracic cavity, may also be collected and imaged both *in situ* and *ex situ* prior to being passed on to pathology.

Collected surgical specimens will be documented by the clinical research coordinator in the Imaging Workbook as called out by the surgeon. The fluorescence status of each specimen will similarly be assessed and called out by the surgeon (fluorescent or not fluorescent). All samples will be sent to pathology for routine analysis. To determine the performance metrics of pegsitacianine, the fluorescence status of each specimen will be correlated to the final pathology and results will be tabulated.

3.2 Imaging Assessments

Imaging assessments will include the calculation [redacted] of all collected surgical specimens (primary tumor, margins, lymph nodes, etc.). Specimens will be imaged both prior to excision and immediately following on a piece of representative normal

tissue outside of the surgical field (all specimens). [redacted] values will be used to further compute [redacted] values for the collected specimens. Correlation of imaging observations to pathological outcomes of each specimen will be used to understand pegsitacianine performance (i.e., PPV, NPV, Sensitivity, Specificity).

3.3 Safety Assessments

Safety assessments will include adverse events (AEs), clinical laboratory test results (hematology and serum chemistry), vital sign measurements (blood pressure, pulse, respiratory rate, and temperature), physical examination findings, and 12-lead electrocardiogram (ECG) results.

4. STUDY DRUG DOSAGE AND ADMINISTRATION

On Day 0 of the study, the patient will receive a mg/kg dose of pegsitacianine The volume of drug and total time of infusion will vary by patient and be reliant on the overall required dose. It is the sponsor's recommendation that the clinical site prophylactically dose the subject with diphenhydramine to reduce the likelihood of the subject experiencing an infusion-related reaction.

4.1 Prohibited Medications and Restrictions

Patients may not take or receive the following:

- [redacted]
- Concomitant medication with a high probability of [redacted] as judged by the Investigator.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. However, it is the responsibility of the principal investigator to ensure that details regarding the medication are recorded in full in the subject's electronic case report form (eCRF). This record will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter medications. Any changes in concomitant medications also will be recorded in the subject's eCRF.

4.2 Study Drug Description

Pegsitacianine is a novel, micelle-based, fluorescent imaging agent. The micelles are comprised of a [redacted]

Pegsitacianine drug product is supplied as a greenish, sterile, frozen, aqueous solution composed of pegsitacianine drug substance formulated as a [redacted] use. The product is limited to investigational use only.

All constituents of pegsitacianine are either used in an FDA approved drug, on an FDA list of constituents of food, or concluded to be safe for ingestion by an expert panel commissioned by the World Health Organization (WHO) (EAFUS, 2018; WHO, 1998).

4.3 Study Drug Safety

The most common and anticipated adverse events related to pegsitacianine include infusion related reactions. These reactions occur in about 1 in 3 patients and tend to be mild or moderate in severity. In general, these reactions initiate within 5 minutes of infusion start and resolve within minutes after onset.

Over the doses evaluated in the Phase 1 ON-1001 study (0.1-1.2 mg/kg), pegsitacianine was well tolerated in patients with solid tumors (HNSCC, BC, CRC, or EC) and showed no dose-limiting toxicity (DLT) (hence the maximum tolerated dose was not reached during the study) or study-drug-related serious adverse events (SAEs). Since no pegsitacianine-related SAEs were reported in Phase 1a, the highest dose from Phase 1a [redacted] was selected for further evaluation of safety, PK, and imaging feasibility of pegsitacianine in Phase 1b in 15 additional patients across 4 tumor types (BC, HNSCC, CRC, EC) at administration [redacted] before surgery (and imaging).

In Phase 1a and 1b, all 30 patients (100%) experienced treatment-emergent adverse events (TEAEs). The most common TEAEs (ie, reported ≥4 patients overall) were procedural pain (21/30 patients, 70%), nausea (6/30 patients, 20%), wound complication (4/30 patients, 13%), and constipation (4/30 patients, 13%). The TEAEs of procedural pain and wound complication could be attributed to the surgical procedures (standard of care). Severe (Grade ≥3) TEAEs, none related to study drug, occurred in 6 (20%) patients. Overall, 3 patients experienced 4 TEAEs (dizziness, headache, pain in jaw, and flushing) considered possibly related to the study drug. All TEAEs considered related to study drug were mild (Grade 1) and experienced by patients in the [redacted] dose cohort. These TEAEs were consistent with typical complications known to occur following surgery, of which the Investigator could not completely rule out the possible contribution of pegsitacianine.

[redacted]

Transient increases in alanine aminotransferase (ALT) (up to 7× upper limit of normal [ULN]), aspartate aminotransferase (AST) (up to 10×ULN), and/or alkaline phosphatase (up to 3×ULN) levels were reported in 4 patients at ≥1 time point after dosing; none of these abnormalities were considered to be related to the study drug; These elevations were judged by the Investigator to be complications due to the length of surgery and the duration of anesthesia.

Apart from 1 moderate (Grade 2) event of hypotension, there were no clinically significant findings with respect to vital signs.

No patient had a suspected unexpected serious adverse reaction (SUSAR), withdrew from the study due to a TEAE, or had an abnormal electrocardiogram (ECG) that was considered clinically meaningful.

In the completed Phase 2 study, a total of 30 patients have been administered pegsitacianine at doses of [redacted] of drug. A total of 26 out of 30 patients (87%) experienced at least one TEAE. In total, 88 TEAEs were documented across the 30 patients. 58 (66%) of the TEAEs were determined to be mild (Grade 1) and all but three TEAEs have resolved. The most commonly encountered TEAE was the sensation of feeling cold, either at the injection site or throughout the body, which was reported for 11 out of the 58 (19%) Grade 1 TEAEs; all TEAEs attributable to feeling cold were resolved.

A total of 7 patients experienced at least one moderate TEAE (23%) (Grade 2) that were either possibly related, probably related, or definitely related to the study drug. The possibly and probably related TEAEs included superficial thrombophlebitis, thrombophlebitis, an infusion related reaction and urticaria, designated as SAEs, as well as instances of lightheadedness and presyncope, thrombophlebitis, an infusion related reaction, itch, and chest tightness that were not considered serious. No medical intervention was required for those patients exhibiting thrombophlebitis, however both these patients were dosed at the highest dose administered (3 mg/kg) and all TEAEs resolved. One subject experienced a Grade 2 infusion related reaction that was attributable to improper preparation of the dose. The second Grade 2 infusion reaction, and the instance of urticaria, were characterized as probably related to the study drug and documented as a SUSARs. The subject experienced flushing, feeling faint, bradycardia, and hypotension within minutes of infusion. Infusion was stopped, the reaction was treated with IV fluids and methylprednisolone, and the event resolved. The patient was monitored in the emergency room of the administrating hospital for approximately 3 hours until cleared to leave the same day. The subject exhibiting urticaria was treated with diphenhydramine (Benadryl) and monitored at the clinic until the symptoms resolved and the patient was cleared to return home.

There were a total of eight Grade 3 TEAEs, all but one instance (high blood pressure, probably related) were not related to the administration of the study drug.

None of the patients enrolled in the ongoing Phase 2 study withdrew from the study due to a TEAE, or had an abnormal ECG that was considered clinically meaningful and related to the study drug.

In the completed Phase 1 healthy volunteer study to evaluate the pharmacokinetics of pegsitacianine, 5 patients of the 17 dosed (29.4%) experienced an infusion related reaction to pegsitacianine. Patients exhibited symptoms that included diaphoresis, lightheadedness, nausea, warmness/flushing, chest heaviness, palpitations, and back pain/spasms. All TEAEs presented shortly after infusion start (3-5 min) and resolved on their own shortly thereafter (3-5 min duration). One subject chose to discontinue the product and withdrew consent. In the remaining patients, the product was halted and restarted in 2 patients with no re-emergence of symptoms while the remaining 3 patients received the complete dose without pause. All TEAEs were deemed to be mild in severity by the investigator.

4.4 Study Drug Packaging and Storage

Frozen vials of pegsitacianine are stored at [redacted]t. Drug product vials are stable for up to [redacted] The final formulation syringes may be prepared and stored at [redacted] from [redacted] prior to administration. The drug product may only be exposed to ambient (room) temperature for up to 4 hours total during the preparation process.

Prior to administration, the frozen formulation is thawed at room temperature to a clear, greenish-colored solution and administered using a syringe pump. The formulation may be thawed at room temperature to obtain a clear solution and stored [redacted] to [redacted] should be used to prime the IV tubing and to flush the agent. All formulations at different stages of administration (frozen, during thawing, and upon preparation in a syringe) are to be protected from light.

4.5 Drug Accountability

The investigator will maintain accurate records of receipt of all test articles, including dates of receipt. In addition, accurate records will be kept regarding when and how much test article is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all study medication will be reconciled and retained or destroyed according to applicable state and federal regulations.

5. SUBJECT ENROLLMENT

5.1 Inclusion Criteria

For inclusion in the study, each subject is required to meet all the following criteria:

1. Adults 18 years of age and older
2. Biopsy confirmed diagnosis, or a high clinical suspicion of a lung malignancy based on MRI, CT and/or PET imaging.
3. Candidates for surgery as determined by the Investigator.
4. Documented negative serum or urine pregnancy test for women of childbearing potential.
5. Male patients and female patients of child-bearing potential (i.e. premenopausal women with intact reproductive organs and women <2 years after menopause) must agree to and comply with using medically acceptable contraception including surgical sterilization (e.g. hysterectomy, bilateral oophorectomy, bilateral tubal ligation), intrauterine device, oral contraceptive, contraceptive patch, long acting injectable contraceptive, partner's vasectomy, double-barrier method (condom or diaphragm plus spermicide or condom plus diaphragm), or abstinence during the trial and for 6 months thereafter
6. [redacted]
7. Agreement to complete all follow-up visits
8. Willing and able to provide written informed consent

5.2 Exclusion Criteria

Any of the following will be regarded as a criterion for exclusion of a subject from the study:

1. [redacted]
2. Tumor locations the surgeon deems unfeasible to image intraoperatively
3. Excessive and/or generalized metastatic disease deemed inoperable by the surgeon
4. Life expectancy less than 12 weeks
5. Karnofsky Performance Status less than 70%
6. Hepatic impairment that in the opinion of the investigator would exclude the subject from the study
7. Lab values that in the opinion of the investigator would prevent surgery
8. Pregnant or lactating
9. Taking or plan to take medications with known hepatoxicity
10. Any other significant medical condition the Investigator deems inappropriate for the trial including any medical or psychiatric conditions that would impair informed consent

5.3 Randomization Procedures

N/A

5.4 Blinding Procedures

Not applicable; this is an open label study.

5.5 Breaking the Blind

Not applicable.

5.6 Subject Withdrawal

Patients are free to withdraw from the study at any time for any reason, without affecting future medical management and treatment.

5.6.1 Reasons for Withdrawal

A subject may be withdrawn from the study by the investigator or OncoNano Medicine Inc for any of the following reasons:

- The subject develops a disease or condition that, in the opinion of the investigator, would compromise the subject's safety by continuing in the study.
- The subject violates the protocol. If, in the opinion of the investigator and OncoNano Medicine, the violation is not likely to impact safety or endpoint evaluations, the investigator may permit the subject to remain in the study at the investigator's

discretion and after approval by OncoNano's medical monitor. Justification for this decision should be clearly documented in source documents.

- The subject becomes noncompliant or becomes uncooperative in returning for the scheduled study visits.
- The subject requests withdrawal for any reason.

The clinical study report will include reasons for subject withdrawals as well as details relevant to the subject withdrawal.

5.6.2 Handling of Withdrawals

If a subject withdraws prematurely from the study after receiving the study drug, study staff should make every effort to perform the Day 21 (±10 days) assessments. The reason for subject withdrawal must be documented in the eCRF.

If a subject withdraws from the study because of an AE (clinical or laboratory), the subject will be asked to return to the clinic for, at a minimum, the evaluations scheduled. If the AE has still not resolved, additional follow-up will be performed as appropriate and documented in the subject's medical records. As a minimum requirement, AEs should be followed for 30 days after receipt of the subject's last dose of study drug, until resolution, or when judged to be stable for 30 days.

If a subject is lost to follow-up, 3 attempts (i.e., 2 phone calls, then 1 registered letter) to contact the subject will be made and documented in the subject's medical records.

5.6.3 Replacements

Withdrawn patients may be replaced at the discretion of the sponsor.

5.6.4 Termination of Study

If, in the opinion of the investigator, the clinical observations in the study suggest that it may be unwise to continue, the investigator may terminate the study after consultation with OncoNano Medicine. A written statement fully documenting the reasons for such a termination will be provided to OncoNano Medicine. In addition, OncoNano Medicine reserves the right to discontinue the study at any time for any reason. Such a termination must be implemented by the investigator, if instructed to do so by OncoNano Medicine, in a time frame that is compatible with patients' well-being.

If the study is terminated, all patients will undergo a complete follow-up examination. Any clinically relevant findings, including clinically significant laboratory values and AEs, will be followed until resolution, stabilization for 30 days, or until the end of the study (the last study evaluation for the last subject), whichever occurs first.

6. STUDY VISITS

6.1 Screening and Enrollment (Days –30 to –1)

Before the study doctor can administer pegsitacianine, he/she must ensure that it is safe for the patient to be in the study vis-à-vis information that is already known about pegsitacianine. After the patient has agreed to be in the study and signed the informed consent form, the study team will perform the following procedures:

- Collect demographic data
- Record their complete medical history
- Perform a complete physical examination
- Measure their weight and height and calculate a body mass index (BMI) to estimate body fat tissue
- Record vital signs (temperature, heart rate, blood pressure, and breathing rate)
- Obtain blood for routine laboratory tests, including a pregnancy test for females who can become pregnant
- Record medications they have been taking, including prescription, over-the-counter, and herbal medications
- Determine Karnofsky Performance Status (to assess the ability to perform ordinary daily tasks)
- Confirm that the patient has met all the study criteria to participate

6.2 Pegsitacianine Administration (Day 0)

Predose procedures

- Confirm eligibility for the study and that medical information is complete
- Record weight
- Discuss how the patient is feeling and their everyday activities
- Record vital signs
- Perform a 12-lead electrocardiogram (ECG) to check the heart activity during pre-operative screening
- Install an IV line
- Obtain blood for routine laboratory tests, including a pregnancy test for females who are capable of becoming pregnant
- Record all medications taken since the Screening Visit, including prescription, over-the-counter, and herbal medications

The Sponsor recommends t[redacted]

Dosing and postdose procedures

Procedures during and after dosing of pegsitacianine are summarized below:

- Dosing of pegsitacianine will be administered
  , length of infusion and overall volume will depend on the assigned dose and the patient's body weight.
- The following postdose assessments and procedures will occur after infusion of the study drug has been completed:
  - Record the patient's vital signs immediately after the infusion and 30 minutes afterward
  - Discuss any new or unexpected changes in how the patient is feeling
  - Record any AEs
  - Record all medications they are taking, including prescription, over-the-counter, and herbal medications

6.3 Surgery and Follow-up Visits

Procedures at the time of surgery

The following procedures will be performed on the day of surgery (before administration of anesthesia and before surgery:

- Record the patient's vital signs
- Obtain blood for routine laboratory tests
- Record all medications being taking, including prescription, over-the-counter, and herbal medications
- Discuss with the patient any new or unexpected changes they are feeling.
- Record any AEs.

Once the patient is under anesthesia, some procedures will occur that would not normally be part of surgery if the patient were not in this study. The SOC surgical procedures will be allowed to continue with the addition of the imaging steps listed below.

- If the primary tumor is not visually apparent using standard approaches, the NIR camera may be used to help locate and guide the resection of the primary tumor.
  - The primary tumor should be imaged prior to excision and following excision on a piece of normal tissue from a distance equal to the operating distance of the camera.
- If the primary tumor is apparent under standard conditions, it will be imaged prior to resection. Additionally, the resected tissue will be imaged using the

intraoperative camera on a section of normal tissue at a distance equal to the operating distance of the camera

- Following the primary tumor resection, margins (both on the excised primary and the affected lung) will be examined using NIR in an effort to detect residual disease
- All SOC lymph nodes to be collected during the operative procedure will be imaged prior to and following excision in a manner identical to the procedures used for imaging the primary resection
- Once the SOC primary tumor resection is complete, the surgeon will examine the thoracic cavity with the intraoperative NIR camera in search of additional fluorescent disease, or lymph nodes that were missed during SOC
- Additional fluorescent lesions, or lymph nodes will be imaged prior to excision, removed and imaged on a section of normal tissue in a manner analogous to the SOC specimens.
- For all resected specimens (including SOC primary tumor, margins, SOC lymph nodes and additional missed disease), fluorescence status and specimen name will be called out by the surgeon for documentation by the clinical research coordinator in the provided Imaging Workbook

Day of Discharge

- Record the patient's vital signs
- Perform a physical examination
- Obtain blood samples for routine laboratory tests
- Record all medications being taking, including prescription, over-the-counter, and herbal medications
- Discuss any new or unexpected changes they are feeling.
- Record any AEs.

Day 21 (±10 days) after dosing

- Record the patient's vital signs
- Perform 12-lead ECG to monitor heart activity
- Perform a physical examination
- Obtain blood samples for routine laboratory tests, including a pregnancy test for females who can become pregnant
- Record all medications being taking, including prescription, over-the-counter, and herbal medications
- Discuss any new or unexpected changes they are feeling.

- Record any AEs

6.4 Early Withdrawal Procedures

If a subject is withdrawn from the study before study completion, all procedures scheduled for the final visit (Day 21 ±10 days) should be performed if possible.

6.5 Training

Extensive training will be provided to all study staff. Initial training will occur at the Site Initiation Visit, followed by on-site training that will take place during the initial surgical procedures with Sponsor Representatives present in the operating room during the procedures. Sponsor Representatives will ensure all study personnel are adequately trained and follow identical procedures to preserve the integrity of the collected data without providing input on the selection of specimens or their fluorescent status, these tasks are are the sole responsibility of the operating surgeon. Additional retraining may take place if necessary throughout the course of the study. Training will include a review of protocol procedures, NIR camera operating instructions, documentation of specimens and their fluorescence status, and upload of source documentation. All training will be documented and stored within the Trial Master File.

7.STUDY ASSESSMENTS

7.1 Demographic Data/Medical History

Demographic data and a complete medical history (including drug, alcohol, and tobacco use, as well as current use of herbal supplements and multivitamins) will be obtained at Screening.

7.2 Physical Examination

A complete physical examination will be performed at Screening, Day of Discharge and on Day 21. The complete physical examination may include the following organ or body system assessments: skin; head; eyes; ears; nose; throat; thyroid; neurological; chest and lungs; cardiovascular; abdomen (liver and spleen); lymph nodes; musculoskeletal; and extremities.

7.3 Weight and Height

Body weight (kg) and height (m) will be measured at Screening and weight will again be collected on Day 0 prior to dosing. The subject's body mass index (BMI) will be calculated at Screening using the following formula:

$$BMI = \frac{Weight\ (kg)}{(Height\ [m])^2}$$

7.4 Vital Sign Measurements

Vital sign measurements (systolic and diastolic blood pressure, pulse, respiratory rate, and temperature) will be obtained at Screening, Day 0 (Pre/Post dose), Day of Surgery, Day of Discharge and Day 21.

Temperature will be documented in degrees Celsius**.**

Blood pressure and pulse will be measured after a resting period of at least 5 minutes in the supine position with a standard mercury sphygmomanometer or an automated oscillometric blood pressure monitor.

7.5 12-lead Electrocardiogram

A standard safety 12-lead ECG will be conducted on Day 0 (Pre-Dose) and on Day 21. Additional ECGs may be performed at the discretion of the investigator. The investigator or designee will be responsible for reviewing the ECG to assess whether the ECG is within the reference limits and to determine the clinical significance of the results. These assessments will be recorded in the eCRF.

7.6 Clinical Laboratory Tests

Clinical laboratory tests (hematology and serum chemistry) will be performed by a Local and/or Central Laboratory. Blood samples will be collected at Screening, on Day 0, Day of Surgery, Day of Discharge and Day 21.

7.6.1 Pregnancy Tests

For female patients, a serum sample or urine dipstick will be collected for the pregnancy test (β-human chorionic gonadotropin) at Screening and blood samples will be collected for pregnancy tests on Day 0, and on Day 21. Negative pregnancy test results will be required for patients to enroll and continue study participation.

7.6.2 Laboratory Measurements

Blood hematology and serum chemistry tests may be performed using the standard panel of each institution (it is not necessary to add analytes if not part of standard panel unless ordered/recommended by treating physician). The following clinical laboratory parameters are an example of what is to be collected:

Clinical Chemistry	Hematology
Total protein	Hemoglobin
Albumin	Hematocrit
Blood urea nitrogen (BUN)	Erythrocyte count (red blood cells)
Calcium (total and ionized)	Differential leukocytes
Creatinine	Platelet count
Creatine kinase	Total leukocytes (white blood cells)
Chloride	
Total bilirubin	
Alkaline phosphatase	
Glucose	
Sodium	
Potassium	
Inorganic phosphate	
Lactate dehydrogenase	
Total Cholesterol	
Gamma-glutamyl transferase	
Alanine aminotransferase (ALT)	
Aspartate aminotransferase (AST)	

8. REPORTING ADVERSE EVENTS

All adverse events (AEs) will be recorded in the eCRF, whether they are observed by the investigator, reported by the subject, observed from laboratory findings, or collected by other means. Adverse events will be monitored beginning on Day 0, following pegsitacianine administration, through the end of the study (Day 28). Adverse events will be graded by the Investigator using a numerical score according to the defined National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 5.0 (2017).

8.1 Definitions

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study treatment. Patients will be instructed to contact the principal investigator if any symptoms develop.

A treatment-emergent AE (TEAE) is defined as any event not present before the first exposure to the study drug or any event already present that worsens in either intensity or frequency following the first exposure to the study drug.

All AEs that occur beginning on Day 0 must be reported in detail in the eCRF and followed to satisfactory resolution or until the principal investigator deems the event to be chronic or the

subject to be stable. The description of the AE will include the onset date, duration, date of resolution, severity, seriousness, etiology, and the likelihood of relationship of the AE to study drug.

A serious adverse event (SAE) is defined as any event that results in any of the following outcomes:

- Results in death
- Is life threatening, i.e., the subject was, in the opinion of the investigator, at risk of death at the time of the event; it does not refer to an event that, hypothetically, might have caused death if it had occurred in a more severe form
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability and/or incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2 Adverse Event Reporting

All AEs reported or observed during the study will be recorded in the eCRF. Information to be collected includes drug treatment, dosage, type of event, time of onset, investigator-specified assessment of severity and relationship to study drug, time of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if the condition deteriorates at any time during the study after the first exposure to study drug, it should be recorded as an AE.

8.3 Assessment of Causality

The investigator’s assessment of an AE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship or association of the study drug in causing or contributing to the AE will be characterized using the following classification and criteria:

- Unrelated: This relationship suggests that there is no association between the study drug and the reported event.
- Unlikely: This relationship suggests the temporal association between the AE and the study drug is such that the study drug is not likely to have any reasonable association with the AE.
- Possible: This relationship suggests that treatment with the study drug caused or contributed to the AE, i.e., the event follows a reasonable temporal sequence from the time of study drug administration and/or follows a known response pattern to the study drug, but could also have been produced by other factors.
- Probable: This relationship suggests that a reasonable temporal sequence of the event with study drug administration exists and, based on the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the study drug seems likely.
- Definite: This relationship suggests that a definite causal relationship exists between the study drug administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

8.4 Assessment of Severity

The intensity of the AE will be rated according to CTCAE criteria (Version 5.0) ranging from Grade 1 to Grade 5. A brief explanation of the grading is provided below:

- **Grade 1** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- **Grade 2** Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
    - Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- **Grade 3** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
    - Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden
- **Grade 4** Life-threatening consequences; urgent intervention indicated.
- **Grade 5** Death related to AE.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

8.5 Serious Adverse Event Reporting

All SAEs must be reported to the [redacted]ail within 24 hours from the time site personnel first learn about the event. Additional follow-up information, when available, should also be sent to [redacted]

8.5.1 SAE Reporting Contact Information

Serious Adverse Event Report Forms must be submitted within 24 hours and should consist of the trial provided Serious Adverse Event Report Form. The following information should be entered into the EDC database at the time the SAE form is submitted: the demographics page(s), the medical history page(s), the AE page(s) and the concomitant medications page(s). If the subject is hospitalized because of, or during the course of an SAE then a copy of the hospital discharge summary should be provided to the [redacted]mail as soon as it becomes available. Withdrawal from the study and all therapeutic measures will be at the discretion of the principal investigator. All SAEs, regardless of relationship to the study drug, will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the subject to be stable.

9. STATISTICAL METHODS

Additional statistical analyses, other than those described in this section, may be performed if deemed appropriate. Details of the analyses are described in the statistical analysis plan.

9.1 Sample Size

The sample size for this study will be 40 subjects. This sample size is based on historical experience with similar pilot studies with the goal of informing power calculations for future studies. This study is not formally powered for hypothesis testing. A sample size of 40 subjects will be sufficient for developing further understanding of the endpoints described herein. Sample size calculations account for non-evaluable and drop-out patients.

9.2 Populations

All subjects who receive any dose of the study drug will be included in the intent to treat population. The efficacy population will consist of all subjects who receive pegsitacianine and have had a minimum of one (1) image collected during their procedure. The safety population will include all subjects who were administered pegsitacianine whether it be a full or partial dose.

9.3 Rate of Clinically Significant Events

Determining the rate of clinically significant events (CSE) detected by pegsitacianine at the subject-level is the primary objective of this study. CSEs will include pegsitacianine detection of primary tumors otherwise undetectable using SOC procedures, detection of occult disease not otherwise known by the surgeon to exist, the detection of a positive surgical margin, and accurate identification of tumor negative SOC biopsies. A list of CSEs and their definitions is provided below.

- Detection of Synchronous Disease – identification of tumor-containing tissue due to pegsitacianine fluorescence, confirmed via histopathological analysis, that otherwise went undetected during pre-operative imaging (PET, CT, MRI) or by the surgeon during SOC surgery.
    - This occurs when a specimen that comes from the thoracic cavity or lymph nodes, is (1) collected via fluorescence and (2) is pathologically-confirmed as a tumor. Note that any specimen collected from the thoracic cavity following SOC will be considered to be collected by fluorescence.

- Detection of Positive Surgical Margin – following the completion of SOC surgery, the surgical fields will be examined for residual fluorescence. Detection of a positive surgical margin would be those specimens collected as a result of residual pegsitacianine fluorescence following the completion of SOC surgery that return a histologically positive for tumor outcome.
    - when a margin specimen (either from the primary tumor or a lung resection margin) (1) is collected via fluorescence and (2) is pathologically-confirmed as a tumor. Note that any lung resection margin collected will be considered to be collected by fluorescence.

- Identification/Localization of the Primary Tumor – during SOC surgery, the surgeon will examine the affected lung to locate the primary tumor. If the primary tumor is not visually apparent using standard imaging methods the surgeon may use the NIR imaging device to aid in localization. If the NIR imaging identifies and/or locates the primary tumor, which was undetectable using SOC approaches, this will be deemed a CSE.
    - This occurs when the primary tumor is not located using SOC approaches, and is instead identified using NIR imaging.

- Tumor Negative SOC Biopsies – during SOC surgery, the surgeon will collect tissue specimens that are suspicious for disease. If those samples do not demonstrate pegsitacianine fluorescence and are found to be devoid of tumor pathologically, those specimens would be included as a tumor negative SOC biopsy.
    - This occurs when any SOC sample (primary tumor, additional SOC specimen, or lymph node) that is collected is found to be negative for tumor, and did not fluoresce during *ex situ* imaging.

The number and proportion (with corresponding two-sided exact 95% confidence intervals) of subjects with any CSE will be reported.

9.4 Imaging Analysis

Diagnostic performance of pegsitacianine will be assessed at the level of the specimen by calculating the *ex situ* sensitivity, specificity, PPV, and NPV of pegsitacianine in detecting tumor containing tissue. The *ex situ* fluorescence status of collected SOC and pegsitacianine guided specimens will be compared to the histological analyses of the collected specimens (which is used to determine each sample's status as true/false positive/negative). For determining these operating characteristics, consider the following definitions:

- False positive is defined as "fluorescence was observed on the specimen but the specimen was not found to have tumor via histological analysis".
- False negative is defined as "no fluorescence was observed on the specimen but the specimen was found to have tumor via histological analysis".
- True positive is defined as "fluorescence was observed on the specimen and the specimen was found to have tumor via histological analysis".
- True negative is defined as "no fluorescence was observed on the specimen and the specimen was found not to have tumor via histological analysis".

The number of specimens in each category (each combination of true/false positive/negative), along with the true/false positive/negative rates (and corresponding exact two-sided 95% confidence intervals) will be reported. These rates will be used to determine pegsitacianine's tumor detection sensitivity, specificity, PPV, and NPV using the following equations:

$$\boldsymbol{Sensitivity} = \frac{\#\ of\ True\ Positive\ Specimens}{\#\ of\ True\ Positive\ Specimens + \#\ of False\ Negative\ Specimens}$$

$$\boldsymbol{Specificity} = \frac{\#of\ True\ Negative\ Specimens}{\#\ of\ True\ Negative\ Specimens + \#\ of False\ Positive\ Specimens}$$

$$\boldsymbol{PPV} = \frac{\#\ of\ True\ Positive\ Specimens}{\#\ of\ True\ Positive\ Specimens + \#\ of\ False\ Positive\ Specimens}$$

$$\boldsymbol{NPV} = \frac{\#\ of\ True\ Negative\ Specimens}{\#\ of\ True\ Negative\ Specimens + \#\ of\ False\ Negative\ Specimens}$$

These rates will be reported along with exact two-sided 95% confidence intervals.

9.5 Quantification of Fluorescence Intensity

Specimen-to-background (SBR) ratios and tumor-to-background (TBR) ratios will also be calculated using white light and NIR images of both SOC and pegsitacianine guided specimens. SBRs will be calculated using *ex situ* images of collected specimens. The equation for calculating SBR is listed below.

$$\boldsymbol{SBR} = \frac{Mean\ Fluorescence\ Intensity\ (Specimen)}{Mean\ Fluorescence\ Intensity\ (Normal\ Tissue)}$$

TBR is calculated in the same way, but refers specifically to samples that are histologically confirmed as tumor.

The mean fluorescence intensity of the specimen, of the background, and the SBR (or TBR) of each specimen will be summarized by the histologically-confirmed tumor status (either "tumor present" or "tumor not present/normal tissue").

9.6 Diagnostic Imaging Sensitivity Analyses

In this pilot study we are assuming that any fluorescence is indicative of tumor presence. To assess how the amount of observed fluorescence (e.g., the SBR or TBR) may affect the diagnostic accuracy of pegsitacianine, a receiver operating characteristic (ROC) analysis will be performed on all specimens using the observed thresholds of SBR and TBR. The corresponding ROC curve will be provided along with the area under the curve (AUC).

There is potential for within-subject correlation to bias our estimates of pegsitacianine's diagnostic accuracy. To assess this issue, an ROC curve will be constructed from a mixed model that clusters specimens by the subject they originated from. We will compare the AUC of the mixed effects ROC curve to the standard ROC curve to assess if within-subject correlation is causing significant bias in our analyses. If it is determined that significant bias has been introduced, additional analyses on secondary endpoints may be performed to assess the diagnostic accuracy of pegsitacianine.

9.7 Safety Analysis

Safety data will be summarized by treatment group with descriptive statistics and frequency tables. In general, continuous data will be summarized by presenting the number of patients, mean, standard deviation, median, minimum, and maximum values. Categorical data will be summarized by presenting the number (frequency) and percentage of patients at each level of response. Subject disposition will be presented and summarized.

Adverse events will be coded using MedDRA, Version 24.0 or higher, by system organ class and preferred term, and a listing of all AEs will be generated. Treatment-emergent AEs (TEAEs), treatment-related TEAEs, TEAEs by intensity, TEAEs that lead to discontinuation of study drug, and SAEs will be summarized by treatment, system organ class, and preferred term.

The clinical laboratory data will be summarized by time point, treatment group, and change from baseline. The values that are below the lower limit or above the upper limit of the reference range will be flagged. Those values or changes in values that are considered clinically significant by the investigator will also be flagged. Repeated or unscheduled results will not be included in the summary statistics but will be included in the individual data listings.

9.8 Interim Analyses

A single, internal interim analysis may occur after approximately 10 patients have been enrolled in an effort to better understand the feasibility and benefit of pegsitacianine imaging during lung malignancy resections. All analyses described herein will be conducted and evaluated by the sponsor. After an overall review of the data, a decision will be made by the sponsor to continue or terminate enrollment based on the interim findings. The Interim analyses will assess the utility of pegsitacianine based on mean fluorescence intensity analysis, calculation of tumor to background ratios, calculation of sensitivity and specificity, safety, and investigator feedback. The decision to continue or terminate is not based off of any individual endpoint and does not correspond to any collection of statistical tests, but instead a synthesis of all available information.

10. DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Security

The study data will be collected electronically. This electronic data capture (EDC) system complies with ICH GCP, GDPR, and the current 21 CFR Part 11 guidance, Electronic Records and Signatures.

10.2 Case Report Forms

As part of the responsibilities assumed by participating in the study, the principal investigator agrees to maintain adequate case histories for the patients treated as part of the research under this protocol. The principal investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories.

MedTrials Inc. will supply the eCRF. All eCRF information is to be filled in. If an item is not available or is not applicable, this fact will be indicated. The eCRF has an electronic audit trail so changes can be made until the investigator signs the eCRF. Each completed eCRF must be reviewed, signed, and dated by the principal investigator at the completion of the study. The completed eCRF will be collected by study monitors as soon as practical after completion. One copy will remain at the site in the principal investigator's files.

10.3 Monitoring of the Study

The clinical monitor, as a representative of MedTrials Inc. and/or OncoNano Medicine, has the obligation to follow the study closely. In doing so, the monitor will visit the principal investigator and study facility at periodic intervals, in addition to maintaining necessary contact through telephone, email, and letter. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the principal investigator and staff.

All aspects of the study will be carefully monitored, by MedTrials Inc., OncoNano Medicine or a designee, for compliance with applicable government regulation with respect to current International Conference on Harmonisation (ICH) harmonised tripartite guideline E6(R1): Good Clinical Practice and current standard operating procedures.

10.4 Inspection of Records

The principal investigator and institutions involved in the study will permit trial-related monitoring, audits, institutional review board (IRB) review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the principal investigator agrees to allow MedTrials Inc., OncoNano Medicine, representatives of OncoNano Medicine, the United States Food and Drug Administration (FDA), and/or other regulatory agency access to all study records.

The principal investigator should promptly notify MedTrials and OncoNano Medicine of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to MedTrials Inc. and OncoNano Medicine.

10.5 Study Record Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with MedTrials Inc. and/or OncoNano Medicine. It is the responsibility of MedTrials Inc. and OncoNano Medicine to inform the principal investigator as to when these documents no longer need to be retained.

11. ADMINISTRATIVE CONSIDERATIONS

The following administrative items are meant to guide the principal investigator in the conduct of the trial but may be subject to change based on industry and government standard operating procedures or working practice documents or guidelines. Administrative changes will be reported to the IRB but will not result in protocol amendments.

11.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission

of the subject (or the subject's guardian), except as necessary for monitoring and auditing by OncoNano Medicine, its designee, the FDA, or the IRB.

The principal investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from OncoNano Medicine or its designee must be obtained for the disclosure of any said confidential information to other parties.

11.2 Institutional Review Board/Ethics Committee Approval

Federal regulations and the ICH guidelines require that approval be obtained from an IRB before participation of human patients in research studies. Before the study onset, the protocol, informed consent, advertisements to be used for subject recruitment, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with ICH E6(R1) will be maintained by the site and will be available for review by OncoNano Medicine or its designee.

All IRB approvals should be signed by the IRB chairman or designee and must identify the IRB name and address, the clinical protocol by title and/or protocol number and the date approval and/or favorable opinion was granted.

The principal investigator is responsible for obtaining continued review of the clinical research at intervals not exceeding 1 year or otherwise specified by the IRB. The principal investigator must supply OncoNano Medicine or its designee with written documentation of continued review of the clinical research.

11.3 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by OncoNano Medicine or its designee. Amendments to the protocol must be submitted in writing to the principal investigator's IRB for approval before patients are enrolled into an amended protocol.

11.4 Informed Consent

A written informed consent in compliance with Part 50 of Title 21 of the Code of Federal Regulations (CFR) shall be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by OncoNano Medicine to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by OncoNano Medicine and/or its designee, if appropriate, before IRB submission. Once reviewed, the consent will be submitted by the principal investigator to their IRB for review and approval before the start of the study. If the informed consent form is revised during the course of the study, all active participating patients must sign the IRB-approved revised form.

Before recruitment and enrollment, each prospective subject will be given a full explanation of the study and allowed to read the approved informed consent form. Once the principal investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form.

The principal investigator shall provide a copy of the original form of the signed informed consent to the subject and/or legal guardian.

11.5 Protocol Violations and Deviations

The principal investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The principal investigator may implement a deviation from or a change of the protocol to eliminate an immediate hazard to trial patients without prior IRB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IRB for review and approval, to OncoNano Medicine for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by OncoNano Medicine and the IRB and agreed to by the principal investigator. Deviations usually have an impact on individual patients or a small group of patients and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the subject, when the subject or principal investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the subject is enrolled without prior approval by OncoNano Medicine, or when there is nonadherence to FDA regulations and/or ICH E6(R1) guidelines.

The clinical monitor will document protocol violations and deviations throughout the course of monitoring visits. The monitor will notify the principal investigator during a visit and/or in writing of all violations and deviations. The IRB should be notified of all protocol violations and deviations in a timely manner.

11.6 Study Reporting Requirements

By participating in this study, the principal investigator agrees to submit reports of SAEs according to the timeline and method outlined in the protocol. In addition, the principal investigator agrees to submit annual reports to their IRB as appropriate. The principal investigator also agrees to provide OncoNano Medicine with an adequate report shortly after completion of the principal investigator's participation in the study.

11.7 Investigator Documentation

Before beginning the study, the principal investigator will be asked to comply with ICH E6(R1) 8.2 and 21 CFR by providing the following essential documents, including but not limited to:

- An original investigator-signed Investigator Agreement page of the protocol.

- An IRB-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or legal guardians.
- IRB approval.
- Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572.
- Curriculum vitae (CV) for the principal investigator and sub-investigators listed on Form FDA 1572. Current licensure must be noted on the CV. They will be signed and dated by the principal investigator at study start-up, indicating that they are accurate and current.
- Financial disclosure information to allow OncoNano Medicine to submit complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigators must provide to OncoNano Medicine a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.
- Laboratory certifications and reference ranges for any local laboratories used by the site, in accordance with 42 CFR 493.

11.8 Study Conduct

The principal investigator agrees that the study will be conducted according to the principles of the ICH E6(R1) guidelines and the principles of the World Medical Association Declaration of Helsinki. The principal investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

11.9 Publications

Following completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, OncoNano Medicine will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. OncoNano Medicine has final approval authority over all such issues.

Data are the property of OncoNano Medicine and cannot be published without prior authorization, but data and publication thereof will not be unduly withheld.

12. REFERENCES

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13. APPENDIX 1: SCHEDULE OF EVENTS

	Screening	Dosing		Treatment		
Evaluation	**Days –30 to –1**	**Day 0 Pre-Dose**	**Day 0 Post-Dose**	**Surgery**	**Day of Discharge**	**Day 21 (± 10 Days)**
Informed consent & subject enrollment	X					
Inclusion/exclusion criteria	X	X				
Pregnancy test[a]	X	X				X
Demographic data	X					
Medical history	X					
Physical examination	X				X	X
Karnofsky Performance Status	X					
Height[b]	X					
Body weight[b]	X	X				
Vital signs[c]	X	X	X[d]	X	X	X
12-lead electrocardiogram		X				X
Concomitant medications	X	X	X	X	X	X
Study drug administration		X[e]				
Adverse events review[f]			X	X	X	X
Serum chemistry	X	X		X	X	X
Hematology[g]	X	X		X	X	X
Surgery				X		
Intraoperative Imaging				X		
Pathology evaluation of surgical specimens				X[h]		

Footnotes

[a] For female patients, a serum sample or urine dipstick for a pregnancy test will be collected at screening and on Day 0 and Day 21.

[b] Body mass index will be calculated at Screening only.

[c] Vital sign measurements will include systolic and diastolic blood pressure, pulse, respiratory rate, and temperature (in degrees Celsius). Blood pressure and pulse will be measured after a resting period of at least 5 minutes in the supine position.

[d] Patient's vital signs are to be recorded immediately after the infusion and 30 minutes afterward

[e] The Sponsor recommends the use of prophylactic diphenhydramine prior to study drug administration, in an effort to reduce the prevalence of infusion-related reactions.

[f] Patients will be assessed for AEs occurring from the time of dosing through Day 21 (±10 days)

[g] Hematology assessments may include hemoglobin, hematocrit, erythrocyte count (red blood cells), differential leukocytes, platelet count, and total leukocytes (white blood cells).

[h] Pathologic analysis of surgical specimens will take place from the conclusion of surgery and up to 10 days after

14. APPENDIX 2: FDA-CLEARED NIR IMAGING DEVICES

NOTE: this list is not comprehensive and does not restrict the use of other FDA-cleared imaging device from use in this trial.

14.1 NOVADAQ SPY Elite

The NOVADAQ SPY Elite system is an active device used for intraoperative fluorescence imaging. The device is developed for open surgery. The device uses visible (white light) and NIR spectra in real-time to visualize circulation and ICG based tumor detection. The device is commercially available and 510(k) cleared by the FDA for surgical use.

14.2 NOVADAQ SPY-PHI

The NOVADAQ SPY-PHI (Portable Handheld Imaging) system is an active device used for fluorescence imaging. The device uses both visible (white light) and NIR spectra, which can be used for visualization of circulation and ICG-based tumor detection during several (surgical or non-surgical) procedures. The device is commercially available and 510(k) cleared by the FDA for surgical use.

14.3 NOVADAQ PINPOINT

The NOVADAQ PINPOINT is an intraoperative laparoscopic fluorescence imaging system, used in conjunction with an injected ICG based fluorescent imaging agent, to assess perfusion and visualize structural anatomy using visible (white light) and NIR during cardiothoracic, gastrointestinal, breast, and other reconstructive surgical procedures.

14.4 da Vinci Firefly

The da Vinci Firefly system (Intuitive Surgical, California, USA) provides real-time NIR guidance through visualization of injectable fluorescence dye. The device uses visible (white light) and NIR spectra in real-time to visualize circulation and ICG based tumor detection. The device is commercially available and FDA-cleared for visual assessment of vessels, blood flow, and related tissue perfusion using NIR imaging.

14.5 Visionsense Iridium

The Visionsense Iridium camera system (Visionsense, Philadelphia, PA, USA) uses 3DHD technology intra-operatively for real-time infrared fluorescence and white light imaging visualization during live surgery to enhance anatomical feature identification.