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 PERITONEAL METASTASES, IN PATIENTS UNDERGOING CYTOREDUCTIVE SURGERY

PROTOCOL NUMBER: ON-1003

Name of Drug:	Pegsitacianine (ONM-100)
Phase of Development:	Phase 2
Sponsor:	OncoNano
Sponsor Contact:	
Project Manager:	
Medical Monitor:	, MD
Version:	3
Date of Protocol:	09SEP2022
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 subjects 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 peritoneal metastases, in patients undergoing cytoreductive surgery

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9/12/2022

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 (Original)	23JUN2021	Original
2	18JAN2022	• Updated the Medical Monitor • Updated the Project Manager • Included use of prophylactic diphenhydramine prior to pegsitacianine dosing • Removal of urinalysis, oral temperature, triglycerides, uric acid and iron tests • Corrected section numbering • Defined mucinous tumors as those that contain ≥50% mucin • Updated "Day 10" follow-up visit to "Day 10 (±5 days) or Day of Discharge (Whatever event occurs first)" • Updated safety information for the completed ON-1002 study • Corrected secondary endpoints • Removed hypothesis testing from sample size calculations • Updated the requirements to open Group 2 to enrollment • Removed Child-Pugh scores from exclusion criteria and restriction on use of investigational products 30 days prior to, and throughout the duration of the study • Included description of site personnel protocol training • Updated information on the intent to treat, efficacy and safety populations • Updated the statistical analysis to account for correlation
3	09SEP2022	• Increased study enrollment size • Updated efficacy population definition • Updated sponsor contact

		• Updated dosing window to 24-72 (± 8) hours • Clarified Screening Timing • Updated consumption of alcohol prior to surgery requirements • Updated imaging and/or biopsy inclusion wording • Updated wording on infusion rate • Update wording on day of surgery labs

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PROTOCOL SUMMARY

Protocol Number	ON-1003
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 peritoneal metastases, in patients undergoing cytoreductive surgery
Phase of Clinical Trial	Phase 2
Indications	Pegsitacianine is indicated as an imaging agent for intraoperative detection of peritoneal metastases originating from intra-abdominal tumors such as ovarian, colorectal, and gastric cancers. Pegsitacianine is administered intravenously as a single dose prior to surgery.
Study Centers	up to 10 centers
Trial Objectives	Primary Objective: The primary ob ective of this study is to determine if administration of pegsitacianine mg/kg) results in the detection of metastatic disease left behind following standard of care surgical resection of peritoneal metastases. Secondary Objectives: Key secondary objectives are to demonstrate an acceptable safety profile, reliable sensitivity, specificity, negative predictive values, and positive predictive values of the imaging agent at the level of the individual specimens.
Background	Pegsitacianine is an intraoperative nanoparticle-based fluorescence imaging agent that was evaluated in the first-in-human Phase 1 study (ON-1001) completed in the Netherlands in patients with solid cancers undergoing surgical excision of their tumors. In addition, it was also evaluated in the 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 low-pH tumor microenvironment. Pegsitacianine was demonstrated to be well tolerated and showed no dose-limiting toxicity (DLT).
Trial Design	This Phase 2 study will be a two Group interventional, open-label, single arm trial where each patient is his/her own "intra atient" control. All atients will receive a single mg/kg dose of pegsitacianine prior to standard of care surgery. It is recommended by the sponsor to administer prophylactic diphenhydramine before administration of pegsitacianine to decrease the possibility of an infusion-related reaction. Patients enrolled in Group 1 of the study will have a biopsy confirmed diagnosis of non-mucinous (i.e., ≤50% mucin) peritoneal carcinomatosis with a suspected Peritoneal Carcinomatosis Index (PCI) greater than or equal to 10. Group 2 of the study will include patients with a suspected PCI greater than or equal to 10 and a biopsy confirmed diagnosis of mucinous (i.e., >50% mucin) peritoneal carcinomatosis. A total of approximately 60 patients will be enrolled across both Groups. Enrollment will be open first to Group 1 with the opportunity to open Group 2 for enrollment following the demonstration of satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Group 1. In both Groups 1 and 2, the surgeon will perform their SOC resection of visible metastases and image up to a total of 15 resected tumor (n = 10) and normal (n=5)

	specimens on an area of normal tissue within the surgical field using the intraoperative NIR camera. The SOC specimens are to be resected upon the initiation of surgery and imaged on a section of normal tissue (i.e., mesentery). Specimen names and fluorescence status will be dictated by the surgeon and documented in the Imaging Workbook by the research coordinator. At the conclusion of SOC resection, the intraoperative NIR camera will be used to investigate the peritoneal cavity further for evidence of residual metastases highlighted by fluorescence. Additional fluorescent specimens will be imaged *in situ* and then *ex situ* on an area of normal tissue within the surgical field prior to sending all surgical specimens to pathology for examination. The fluorescence status of each specimen will then be directly correlated to the histopathological outcomes of each specimen. This study design enables the calculation of pegsitacianine sensitivity, specificity, NPV, and PPV. Additionally, Completeness of Cytoreduction (CC) scores can be calculated following SOC resection and adjusted following the resection of additional fluorescent metastases, if warranted.
Endpoints	<u>Primary Endpoints:</u> • Detection of disease left behind following SOC resection of peritoneal metastases. Detection of a single additional tumor-containing specimen excised as a result of pegsitacianine fluorescence will be deemed a clinically significant event (CSE) • Alteration to initial Completeness of Cytoreduction scores following imaging with pegsitacianine <u>Secondary Endpoints:</u> • Acceptable safety profiles following pegsitacianine administration • Sensitivity • Specificity • NPV • PPV
Study Drug	m /k of e sitacianin prior to surgery
Eligibility	<u>Inclusion Criteria:</u> 1. Adults 18 ears of a e and older 2. [redacted] 3. [redacted] ≥10) 4. Acceptable hematologic status, kidney function and liver function (as standard surgery protocol requires), as determined by the Investigator. 5. Documented negative serum pregnancy for women of childbearing potential 6. 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 7. Agreement to abstain from heavy alcohol consumption (>4 drinks/day) 72 hours prior to administrations and for minimum of 10 days post-surgery 8. Agreement to complete all follow-up visits 9. Willing and able to provide written informed consent Exclusion Criteria: 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. Achieving [redacted] is deemed unlikely by the surgeon 5. Life expectancy less than 12 weeks 6. Karnofsky Performance Status less than 70% 7. Lab values that in the opinion of the investigator would prevent surgery 8. Medical or psychiatric conditions that would impair informed consent 9. Pregnant or lactating 10. Taking or plan to take medications with known hepatoxicity 11. Any other significant medical condition the investigator deems inappropriate for the trial
Sample Size	N=60 Approximately 60 patients will be enrolled in this study. The number of patients per Group will be reliant on the descision to activate Group 2. No set allocation of patients to either group will be implemented, rather enrollment will be open first to Group 1 with the opportunity to open Group 2 for enrollment following the demonstration of satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Group 1.
Statistical Analysis	Proportion of patients for whom an additional metastatic deposit was found and confirmed to be tumor using florescence imaging will be calculated by determining the quotient of subjects with a CSE over the total number of patients undergoing surgery who have received >75% of the intended dose of pegsitacianine and have sufficient imaging data. Sensitivity and specificity will be determined at the specimen level using correlated fluorescence observations and pathological outcomes.
Schedule of Events	**Screening (Day -30 to 0)** 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: • 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 a blood sample for routine laboratory tests, including a pregnancy test for females who are capable of becoming pregnant
- Record medications they have been taking, including prescription, over-the-counter, and herbal medications
- 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 and height and calculate BMI
- 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 are capable of becoming pregnant
- Obtain a blood, urine or breath test to test for alcohol. This test may be performed if relevant to the patient's medical history. If a breath/urine test is used and is positive, a blood test may be used to confirm the test results
- Record all medications taken since the Screening visit, including prescription, over-the-counter, and herbal medications

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:

- Dosin of e sitacianine will be administered . Total dosing time will be dependent on the the patient's body weight and total volume to be administered
- 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 60 minutes afterward
  - Discuss any new or unexpected changes in how the patient is feeling
  - Record all medications they are taking, including prescription, over-the-counter, and herbal medications

Peritoneal Cytoreductive Surgery ; Groups 1 and 2)

Procedures at the time of cytoreductive surgery

The following procedures will be performed on the day of surgery:

- Record the patient's vital signs (only if they were abnormal on Day 0)
- Obtain a blood sample for routine laboratory tests
- Record all medications being taken, including prescription, over-the-counter, and herbal medications
- Discuss with the patient any new or unexpected changes in they are feeling

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.

- During SOC resection, up to 15 specimens (10 suspected tumor and 5 normal) specimens will be imaged using the intraoperative camera on a section of normal tissue
- Once the SOC resection is complete, the surgeon will examine the peritoneal cavity with the intraoperative NIR camera in search of additional fluorescent metastatic disease that was missed during SOC using an assessment method similar to that used for establishing the PCI score (i.e., 13 regions)
- Additional fluorescent lesions will be removed and imaged on a section of normal tissue in a manner analogous to the SOC specimens

Day 10 (±5 days) or Day of Discharge (Whichever event occurs first)

- 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
- Record all medications being taken, including prescription, over-the-counter, and herbal medications
- Discuss any new or unexpected changes or adverse events

Day 28 (±5 days) after dosing

- Obtain blood samples for routine laboratory tests, including a pregnancy test for females who are capable of becoming pregnant
- Record all medications they are taking, including prescription, over-the-counter, and herbal medications
- Discuss new or unexpected changes in they are feeling
- Record vital signs (temperature, heart rate, blood pressure, and breathing rate)

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 occurs during initial surgical procedures with Sponsor Representatives present. Sponsor Representatives will ensure all study personnel are well 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 to the database. 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
ARE	antioxidant response element
AST	aspartate aminotransferase
BMI	body mass index
BUN	blood urea nitrogen
CC	Completeness of Cytoreduction Score
CFR	Code of Federal Regulations
CS	cytoreductive surgery
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
EAFUS	Everything added to food in the U.S.
FDA	United States Food and Drug Administration
HIPEC	Hyperthermic intraperitoneal chemotherapy
HNSCC	head and neck squamous cell carcinoma
ICG	indocyanine green
ICH	International Conference on Harmonisation
IRB	institutional review board
IV	intravenous
MFI	mean fluorescence imaging
MRI	magnetic resonance imaging
NIR	near-infrared
NSCLC	non small cell lung carcinoma
NPV	negative predictive value
PC	peritoneal carcinomatosis
PCI	peritoneal carcinomatosis index
PEG	polyethylene glycol
PET	positron emission tomography
PK	pharmacokinetics

ABBREVIATION	TERM
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
TEAE	treatment-emergent adverse event
WHO	World Health Organization
WMA	World Medical Association

1. INTRODUCTION

1.1 Overview

Peritoneal carcinomatosis (PC) results from the metastasis of a primary cancer of the peritoneum (e.g., appendiceal, ovarian, uterine, colorectal, and gastric cancers) that then disseminates throughout the abdominal cavity.[1] Historically, progression to PC was considered terminal and resulted in survival times on the scale of a few months with palliative care being the best option for patients.[2] More recently, cytoreductive surgery (CS) has emerged as a means to prolong and improve patient lives with a median increase in survival of up to ~5 years.[3] It has been reported that for every 10% increase in cytoreduction there is a 5.5% increase in median survival time.[4,5] In addition to surgical tumor debulking within the peritoneal space, it has also been shown that coupling surgical intervention with hyperthermic intraperitoneal chemotherapy (HIPEC) can have an even greater impact on patient outcomes.[6]

The extent of survival benefit gained via CS is directly linked to the completeness of cytoreduction, or how much macroscopic disease (>2.5 mm deposits) has been removed.[7,8] During CS, surgeons examine the peritoneal cavity for evidence of disease with the intent of removing all visible tumor deposits, and thus providing a complete cytoreduction. Any tumor deposits left behind, smaller than 2.5 mm, are believed to be treated efficiently with the follow-on HIPEC treatment.[9] These surgical procedures are lengthy in duration, with total CS and HIPEC treatment times taking upwards of 14 hours to complete. Additional intraoperative tools for the identification of metastatic deposits may help to reduce total surgical time, as well as help identify disease that may be microscopic or unable to be detected with the naked eye (i.e., deep metastases). In an effort to aid surgeons in disease detection, focus has been placed on the development of fluorescent imaging agents that can accurately detect small deposits of disease that may be missed under standard white light surgical procedures.[10,11]

One strategy to address the challenges that physicians face during surgery, and to overcome the complexity encountered due to the diversity in oncogenotypes and histologic phenotypes, is to target metabolic vulnerabilities that are ubiquitous in cancer. Aerobic glycolysis, known as the Warburg effect, in which cancer cells preferentially take up glucose and convert it into lactic acid, occurs in all solid cancers.[12] Lactic acid, a by-product of cellular metabolism, accumulates in the extracellular space through excretion from tumor cells via the action of monocarboxylate transporters.[13] The resulting acidification of the extra-cellular space promotes remodelling of the extracellular matrix for further tumor invasion and metastasis.[14]

Pegsitacianine, a micellar fluorescence imaging agent, exploits the ubiquitous pH differences observed between cancerous and normal tissues. This in turn, provides a highly sensitive and specific fluorescence response after localizing within the tumor microenvironment, thus allowing the detection of primary tumors, their margins, metastatic disease, and tumor-containing lymph nodes.[15-17]

There exists a clear unmet clinical need for the intraoperative detection of tumor margins, metastatic disease and cancer-involved nodes. Solutions to these challenges would allow surgeons to more precisely identify and remove primary cancers or metastases, and selectively remove only those lymph nodes in which disease is present. In addition to enabling a more comprehensive removal of malignant disease, the use of technologies, such as pegsitacianine, would help to reduce the morbidity of radical surgery by reducing the excisional volume of normal tissue and overall surgical time.[18]

1.2 Pegsitacianine Clincal Trial Background

Three clinical studies have been performed 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.[19] 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.

The purpose of the ON-1001 study was to investigate the safety, pharmacokinetics and feasibility of pegsitacianine as an intraoperative optical tracer 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 micelle dose was administered intravenously (IV) as a one to five-minute infusion to patients in five dose cohorts (0.1, 0.3, 0.5, 0.8, and 1.2 mg/kg) with three patients per cohort in Phase 1A, and 15 patients at a dose of 1.2 mg/kg 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 also imaged postoperatively. The median tumor-to-background (TBR) value from all bread loaf slice image analysis (n = 97 slices from 27 patients) was determined to be 4.5 with an interquartile range of 3.1. Additionally, pegsitacianine demonstrated a sensitivity of 100% across all tumor types tested with a specificity of 75% and 57% in breast cancer and head and neck squamous cell carcinoma (HNSCC), respectively.[19]

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 24 ±8 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.

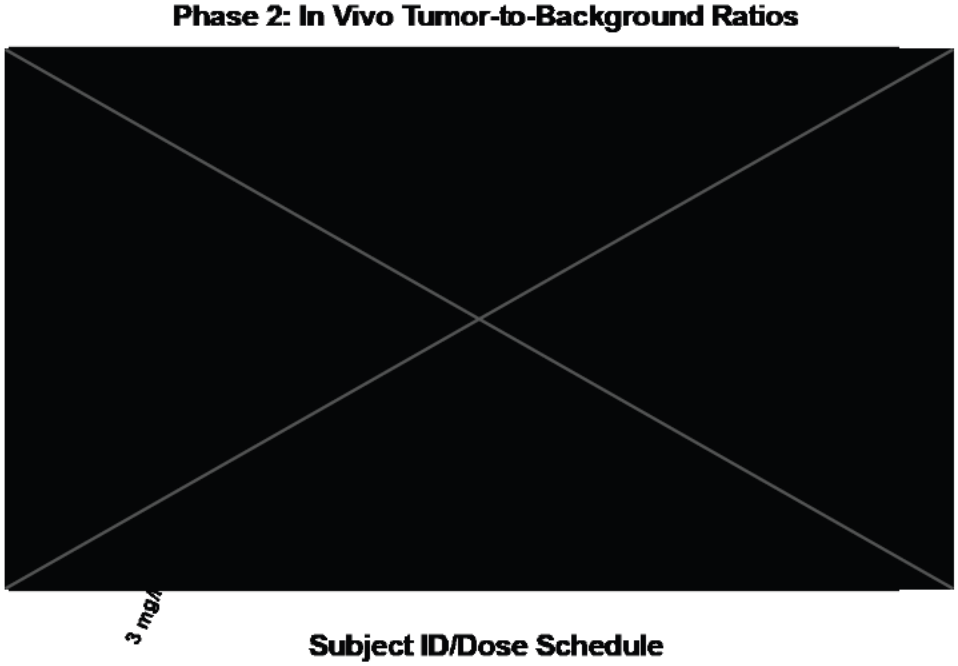
Part 3 was used to assess the safety and efficacy (sensitivity and positive predictive value [PPV]) of pegsitacianine at a dose of mg/kg for intraoperative imaging during HNSCC surgery, administered at [redacted] hours prior to surgery, in addition to a set of secondary and exploratory endpoints to further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

ON-1004 was a Phase 1 study conducted in normal, healthy volunteers. The study is 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 tumor-to-background (TBR) ratios for subjects receiving [redacted] were not significantly different across the two studies to date. (The red line represents the average TBR calculated across all patients in each study).

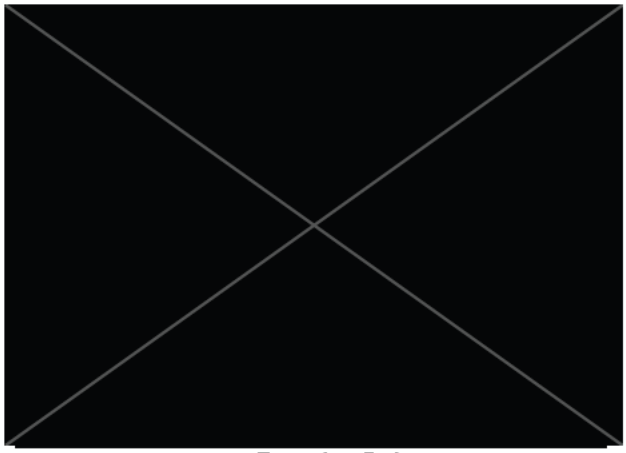
All dose levels investigated in the ON-1001 Phase 1 Trial were imaged 24±8 hours following the completion of the pegsitacianine infusion



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 subjects 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 mg/kg was chosen as the ideal dose for the continued evaluation of pegsitacianine in the completed ON-1002 study, as well as the proposed ON-1003 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 metastatic disease left behind following standard of care surgical resection of peritoneal metastases.

2.2 Secondary Objectives

Key secondary objectives are to demonstrate an acceptable safety profile, reliable sensitivity, specificity, negative predictive values, and positive predictive values of the imaging agent at the level of the individual specimens

2.3 Exploratory Objectives

Assess the how the normalized SBR value 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 a an interventional, open-label, single arm trial where each atient is his/her own "intrapatient" control usin two Grou s. All atients will receive a single mg/kg dose of pegsitacianine rior to standard of care surgery.

Patients enrolled in Group 1 of the study will have a biopsy confirmed diagnosis of non-mucinous peritoneal carcinomatosis, defined as less than, or equal to 50% mucin in the sample, with a suspected Peritoneal Carcinomatosis Index (PCI) greater than or equal to [redacted]. Group 2 of the study will include patients with a suspected PCI greater than or equal to [redacted] and a biopsy confirmed diagnosis of mucinous peritoneal carcinomatosis, defined as > 50% mucin in the sample. A total of approximately 60 patients will be enrolled across both Groups. Enrollment will be open first to Group 1 with the opportunity to open Group 2 for enrollment following the demonstration of satisfactory pegsitacianine sensitivity and specificity values of 70% or greater in Group 1.

In both Groups 1 and 2, the surgeon will perform their SOC resection of visible metastases and image each resected specimen, and normal specimens on an area of normal tissue using the intraoperative NIR camera; no investigative NIR imaging of the peritoneal cavity will be performed at this time. The SOC specimens are to be resected upon the initiation of surgery and imaged on a section of normal tissue (i.e., mesentery). Specimen names and fluorescence status will be dictated by the surgeon and documented in the Imaging Workbook by the Clinical Research Coordinator.

At the conclusion of SOC resection, the intraoperative NIR camera will be used to investigate the peritoneal cavity further for evidence of residual metastases highlighted by fluorescence using the approach that is performed for the calculation of the Peritoneal Carcinomatosis Index (i.e., 13 regions). Additional fluorescent specimens will also be imaged prior to excision and subseqently following resection on an area of normal tissue prior to sending all surgical specimens to pathology for examination. The fluorescence status of each specimen, as called out by the surgeon, will then be directly correlated to the histopathological outcomes of each specimen dictated in the final pathology report. This study design enables the calculation of pegsitacianine sensitivity, specificity, NPV, and PPV and the level of the specimens. Additionally, Completeness of Cytoreduction (CC) scores can be calculated following SOC resection and then adjusted following the additional resection of fluorescent metastases otherwise unknown to the surgeon. The schedule of events for this study is presented in Appendix 1.

3.2 Imaging Assessments

Imaging assessments will include the calculation of mean fluorescence intensity (MFI) of all collected surgical specimens. Specimens will be imaged both prior to excision (for post-SOC specimens) and immediately following on a piece of representative normal tissue (all specimens). MFI values will be used to further compute SBR 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). Imaging assessments may be performed at the Group level to accommodate for any confounding data between the two Groups.

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 oral temperature), physical examination findings, and 12-lead electrocardiogram (ECG) results.

4. STUDY DRUG DOSAGE AND ADMINISTRATION

On Da 0 of the stud the atient will receive a mg/kg dose of pegsitacianine The volume of drug and rate of infusion will vary by patient and be reliant on the overall required dose.

4.1 Prohibited Medications and Restrictions

Subjects may not take or receive the following:

- [redacted]
- Concomitant medication with a high probability of [redacted], as judged by the PI

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

Pe sitacianine is a novel micelle-based fluorescent ima in a ent. The micelles are comprised of a [redacted] conjugated to ICG, an FDA-approved fluorophore.

Pegsitacianine drug product is supplied as a greenish sterile frozen a ueous solution com osed of e sitacianine dru substance formulated as a [redacted]). Each single-use vial contains 3 mL of pegsitacianine Injection for IV use. The product is limited to investigational use only.

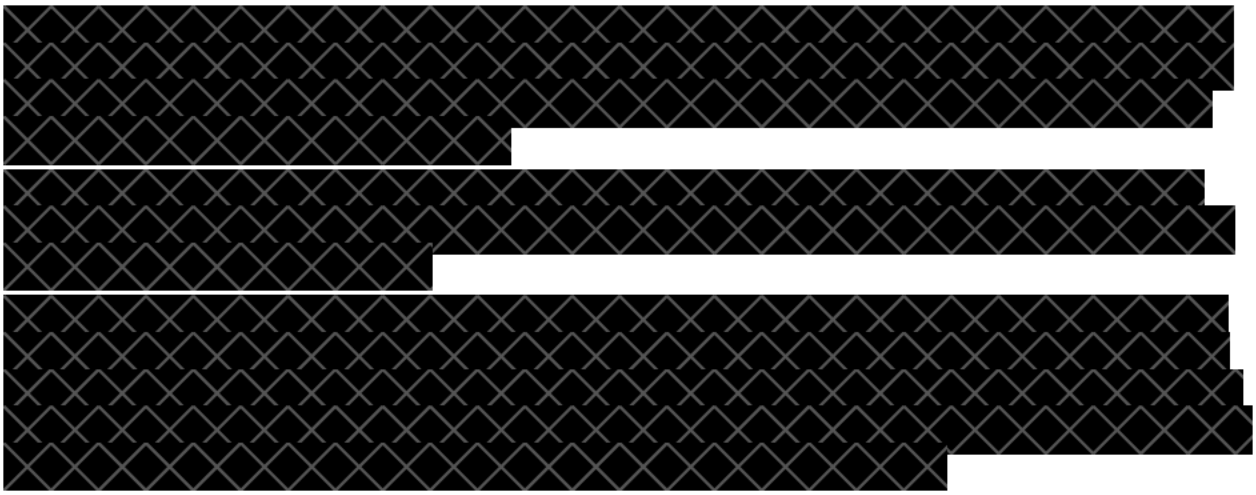
All constituents of pegsitacianine are either used in an FDA approved drug, on an FDA list of constituents of food, or concluded to be safe for ingestion by an expert panel commissioned by the World Health Organization (WHO) (EAFUS, 2018; WHO, 1998).

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 (1.2 mg/kg) 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 24 (±8) hours before surgery (and imaging).

[redacted]



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 1, 2, or 3 mg/kg with surgery occurring between 3 and 24 hours post-administration of drug. A total of 26 out of 30 subjects (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 subjects 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, presyncope, thrombophlebitis, an infusion related reaction, itch, and chest tightness that were not considered serious. No medical intervention was required for the subjects exhibiting thrombophlebitis, however both these subjects 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 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 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.

4.4 Study Drug Packaging and Storage

Frozen vials of pegsitacianine are stored at [redacted]. Drug product vials are stable for up to 24 months (stability testin on oin [redacted] The final formulation s rin es ma be re ared and stored at [redacted]

Prior to administration the frozen formulation is thawed at room tem erature to a clear reenish-colored solution

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 study drug, 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 of the following criteria:

1. Adults 18 ears of a e and older
2. [redacted]

[redacted]

3. Suspected Peritoneal Carcinomatosis Index greater than or equal to [redacted]
4. Acceptable hematologic status, kidney function and liver function (as standard surgery protocol requires), as determined by the Investigator
5. Documented negative serum pregnancy for women of childbearing potential
6. 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
7. Agreement to abstain from heavy alcohol consumption (>4 drinks/day)72 hours prior to administrations and for minimum of 10 days post-surgery
8. Agreement to complete all follow-up visits
9. 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. Known h__ersensitivit__ or aller__ to indoc_anine__reen_ICG [redacted]
2. Tumor locations the surgeon deems unfeasible to image intraoperatively
3. Excessive and/or__eneralized metastatic disease deemed ino_erable b__the surgeon
4. [redacted] is deemed unlikely by the surgeon
5. Life expectancy less than 12 weeks
6. Karnofsky Performance Status less than 70%
7. Lab values that in the opinion of the investigator would prevent surgery
8. Medical or psychiatric conditions that would impair informed consent
9. Pregnant or lactating
10. Taking or plan to take medications with significant risk of hepatoxicity
11. Any other significant medical condition the investigator deems inappropriate for the trial

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

Subjects 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 for any reason, study staff will make every effort to perform the Day 28 (±5 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 subjects 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 subjects' well-being.

If the study is terminated, all subjects 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 0)

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:

- 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 are capable of becoming pregnant
- Record medications they have been taking, including prescription, over-the-counter, and herbal medications
- Determine Karnofsky Performance Status (to asses the ability to perform ordinary daily tasks)
- Confirm that the patient has met all the study criteria to participate

6.2 Pegsitacianine Administration (Days 0)

Predose procedures

- Confirm eligibility for the study and that medical information is complete
- Record weight and height and calculate BMI
- 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
- Obtain blood, urine, or a breath test to test for alcohol. This test may be performed if relevant to the patient's medical history. If a urine or breath test is used and is positive, a blood test may be used to confirm the test results
- Record all medications taken since the Screening visit, including prescription, over-the-counter, and herbal medications

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 through an IV injection Total dosing time will depend on the patient's body weight and total volume to be administered
- 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 60 minutes afterward
    - Discuss any new or unexpected changes in how the patient is feeling
    - Record all medications they are taking, including prescription, over-the-counter, and herbal medications

6.3 Surgery and Follow-up Visits (Group 1 and 2)

Procedures at the time of cytoreductive surgery

The following procedures will be performed on the day of surgery:

- Record the patient's vital signs (only if they were abnormal at dosing)

- Obtain blood for routine laboratory tests
- Record all medications being taken, 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 steps listed below.

- During SOC resection, the surgeon will select up to 15 (10 suspected tumor and 5 normal) specimens that will be imaged using the intraoperative camera on a section of normal tissue following excision (*Note: NIR camera should not be used to examine the peritoneal cavity at this time)*
  - A total of up to 15 specimens are to be imaged: 5 representative normal pieces of tissue and 10 individual suspected tumor specimens
  - The SOC specimens are to be resected upon the initiation of surgery and imaged on a section of normal tissue (i.e., mesentery) immediately following the collection of all SOC samples
  - The width of the camera's field of view is to be confined to a distance of 10 cm. A surgical ruler is to be placed in frame to control the width of the field of view
    - Note: while there are no specimen size stipulations provided in this protocol, the samples must be able to be imaged within the 10 cm wide imaging window.
  - For samples with multiple nodules, individual nodules should be dissected away from the bulk sample and imaged individually as single specimens.
    - Representative normal sections of tissue should be imaged in an analogous manner
- Samples will be imaged using the NIR camera and fluorescence status and specimen name will dictated by the surgeon for documentation by the clinical research coordinator in the provided Imaging Workbook
- Once the SOC resection is complete, the surgeon will dictate their completeness of cytoreduction score to the clinical research coordinator for documentation prior to examining the peritoneal cavity with the intraoperative NIR camera in search of additional fluorescent metastatic disease that was missed during SOC
  - The peritoneal cavity will be evaluated using a manner similar to that used for

the calculation of the Peritoneal Carcinomatosis Index. Specifically, the peritoneal cavity will be examined in a methodical manner using the 13 described regions used for calculating the PCI score

- If additional fluorescent areas are to be collected, an image of the additional fluorescent lesion(s) is to be captured prior to excision from the patient
  - The camera should be held 15 cm away from the area of tissue to be excised. The camera's distance can be measured using an available surgical ruler
- Additional fluorescent lesions will then be removed and imaged on a section of normal tissue in a manner analogous to the SOC specimens with the surgeon dictating fluorescence status and specimen name to the clinical research coordinator for documentation
  - The width of the camera's field of view is to be confined to a distance of 10 cm. A surgical ruler is to be placed in frame to control the width of the field of view

Day 10 (±5 days) or Day of Discharge (Whichever event occurs first)

- 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
- Record all medications being taken, including prescription, over-the-counter, and herbal medications
- Discuss any new or unexpected changes they are feeling.
- Record any AEs

Day 28 (±5 days) after dosing

- Obtain blood samples for routine laboratory tests, including a pregnancy test for females who are capable of becoming pregnant
- Record all medications they are taking, including prescription, over-the-counter, and herbal medications
- Discuss new or unexpected changes they are feeling
- Record the patient's vital signs

6.4 Early Withdrawal Procedures

If a subject is withdrawn from the study before study completion, all procedures scheduled for the final visit (Day 28 ±5 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 and on Day 10 (±5 days) or Day of Discharge. 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 at Day 0 prior to dosing. The subject's body mass index (BMI) will be calculated in the eCRF 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 oral temperature) will be obtained at Screening, Day 0 (Pre/Post dose), Day of Surgery (only if abnormal at dosing), Day 10 (±5 days) or Day of Discharge and Day 28.

Oral 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 10 (±5 days) or Day of Discharge. 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 10 (±5 days) or Day of Discharge and on Day 28.

7.6.1 Pregnancy Tests

For female subjects, a serum sample will be collected for a serum pregnancy test (β-human chorionic gonadotropin) at Screening and blood samples will be collected for pregnancy tests on Day 0, and on Day 28. Negative pregnancy test results will be required for subjects to enroll and continue study participation.

7.6.2 Laboratory Measurements

The following clinical laboratory parameters will be measured:

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 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. Subjects 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 as mild, moderate, or severe using the following criteria:

- Mild: These events require minimal or no treatment and do not interfere with the subject's daily activities
- Moderate: These events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning
- Severe: These events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating

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 email within 24 hours from the time site personnel first learn about the event. Additional follow-up information, when available, should also be sent to .

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 co of the hospital discharge summary should be provided to the email 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 was originally 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. While the original sample size of 40 subjects would be sufficient for developing further understanding of the endpoints described herein, increasing the amount of subjects to approximately 60 will provide superior estimates for endpoints to power future studies. 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 >75% of the intended dose of pegsitacianine, had a minimum of one (1) image collected during their procedure, and had the opportunity for post-SOC exploration of the peritoneal cavity. 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

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 Occult Disease or Positive Surgical Margins– identification of tumor-containing tissue due to pegsitacianine fluorescence, confirmed via histopathological analysis, that otherwise went undetected during pre-operative imaging (PET, MRI) or by the surgeon during SOC surgery
    - This occurs when a sample that (1) exhibits fluorescence *in situ* and (2) is pathologically-confirmed as a tumor, is obtained by fluorescence during SOC surgery.

- 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 a sample that (1) exhibits no fluorescence *ex situ* and (2) is pathologically-confirmed as normal tissue, is classified as suspicious tissue during SOC surgery.

- Alteration to the Completeness of Cytoreduction (CC) Score – following the completion of the SOC surgery, the surgeon will document a CC score prior to fluorescence imaging using pegsitacianine. If the surgeon finds residual disease using fluorescence imaging that results in the increase of the CC score, this will be considered a CSE

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:

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

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

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

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

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 subjects, mean, standard deviation, median, minimum, and maximum values. Categorical data will be summarized by presenting the number (frequency) and percentage of subjects at each level of response. Subject disposition will be presented and summarized.

Adverse events will be coded using MedDRA, Version 23.0 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

None planned.

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 subjects 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.

OncoNano Medicine 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 in a timely manner. 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 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 OncoNano Medicine or its 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 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 OncoNano Medicine of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to 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 OncoNano Medicine. It is the responsibility of 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 subjects 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 subjects 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 subjects 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 subjects 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 subjects or a small group of subjects 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 subinvestigators 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.

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13. APPENDIX 1: SCHEDULE OF EVENTS (GROUPS 1 AND 2)

	Screening	**Treatment**			
Evaluation	**Days –30 to 0**	**Day 0**	**Sur er postdose)**	**Day 10 (±5 days) or Day of Discharge**	**Day 28 (± 5 days)**
Informed consent & subject enrollment	X				
Inclusion/exclusion criteria	X	X			
Blood/Urine/Breath alcohol		X			
Pregnancy test[a]	X	X			X
Demographic data	X				
Medical history	X				
Physical examination	X			X	
Karnofsky Performance Status	X				
Height[b]	X	X			
Body weight[b]	X	X			
Vital signs[c]	X	X	X[d]	X	X
12-lead electrocardiogram		X[e]		X	
Concomitant medications	X	X	X	X	X
Study drug administration		X[f]			
Adverse events review[g]		X	X	X	X
Serum chemistry	X	X	X	X	X
Hematology[h]	X	X	X	X	X
Surgery			X		
Intraoperative Imaging			X		
Pathology evaluation of surgical specimens			X[i]		

Footnotes

[a] For female subjects, a serum sample for a serum pregnancy test will be collected at screening and on Day 0 and Day 28.

[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] Vitals are only to be collected if they are found to be abnormal at dosing

[e] 12-lead ECG is to be performed prior to study drug administration

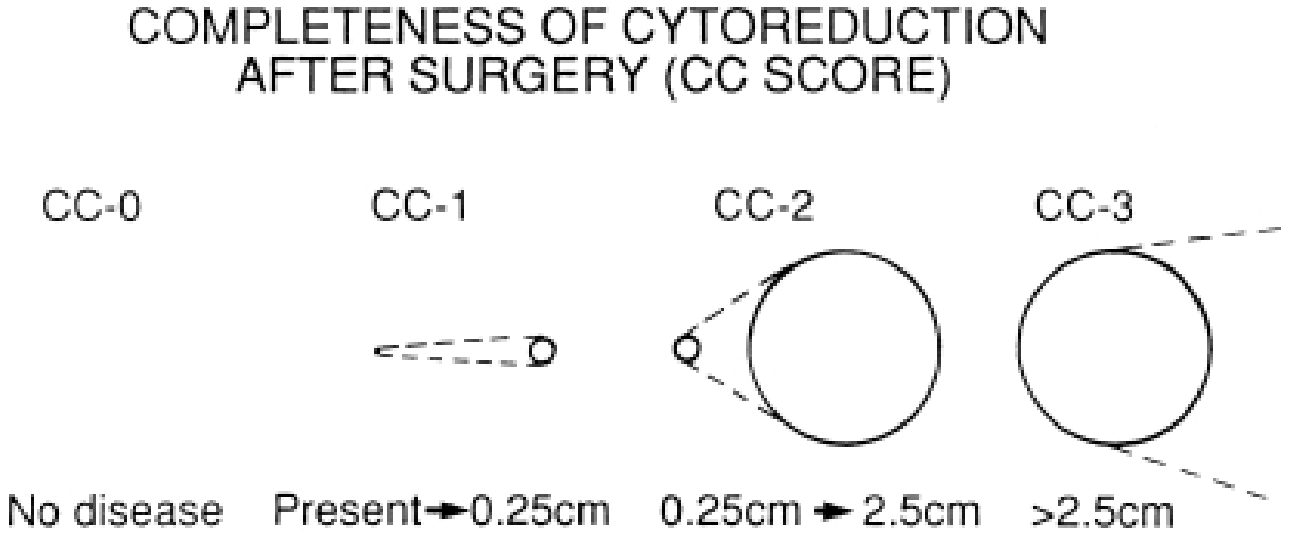
[f] Prophylactic diphenhydramine may be administered prior to the administration of pegsitacianine

[g] Patients will be assessed for AEs occurring from the time of dosing through Day 28 (±5 days)

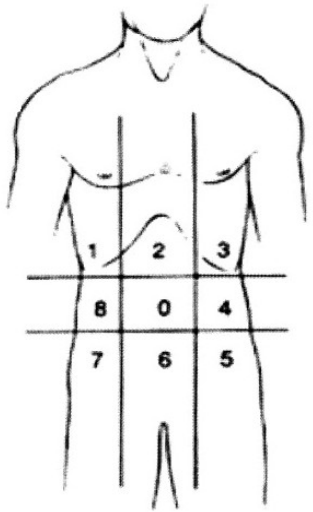
[h] Hematology assessments will include hemoglobin, hematocrit, erythrocyte count (red blood cells), differential leukocytes, platelet count, and total leukocytes (white blood cells).

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

14. APPENDIX 2: COMPLETENESS OF CYOTOREDUCTION SCORE



15. APPENDIX 3: PERITONEAL CARCINOMATOSIS INDEX



Regions	Lesion Size
0 Central	____
1 Right Upper	____
2 Epigastrium	____
3 Left Upper	____
4 Left Flank	____
5 Left Lower	____
6 Pelvis	____
7 Right Lower	____
8 Right Flank	____
9 Upper Jejunum	____
10 Lower Jejunum	____
11 Upper Ileum	____
12 Lower Ileum	____
PCI	☐

Lesion Size Score

LS 0 No tumor seen
LS 1 Tumor up to 0.5 cm
LS 2 Tumor up to 5.0 cm
LS 3 Tumor > 5.0 cm or confluence