[redacted]

# STATISTICAL ANALYSIS PLAN

## Protocol: ON-1005

## 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

**SAP Version:** Version 3

**SAP Date:** 07 NOV 2022

CT Registry Number: NCT04950166

Prepared by

[redacted]

# DOCUMENT HISTORY

## Revision History

| Version Number /Date | | Summary of Changes |
|---|---|---|
| **SAP** | **Protocol** | |
| Ver 1.0<br>23 Nov 21 | Ver 1.0<br>29 Aug 21 | Initial Approved |
| Ver 2.0<br>27 Jan 22 | Ver 2.0<br>25 Jan 22 | • Clarified exploratory objective of SBR effect on diagnostic accuracy<br>• Added ITT population and clarified efficacy population definition<br>• Clarified sample size justification<br>• Clarified definitions of Sensitivity, Specificity, PPV, NPV<br>• Explained ROC analysis and sensitivity analysis for potential within-subject bias |
| Ver 3.0<br>07 Nov 22 | Ver 2.0<br>25 Jan 22 | • Replaced Brian Madajewski with Madeline Olson as a signee.<br>• Acknowledged the Part 2 of the study was not performed.<br>• Updated the efficacy population to require patients receive at least 75% of the dose and had the opportunity for post-SOC evaluation of the thoracic cavity.<br>• Changed comparison of camera type to use the Breslow Day test for homogenous odds ratios.<br>• Added a summary for infusion related reactions. |

## SIGNATURES OF APPROVAL

**Study Title:** 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

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Author: [redacted]

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Reviewer: [redacted]

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Approver: [redacted]

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## LIST OF 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 |
| IRR | Infusion Related Reaction |
| 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 |

| ABBREVIATION | TERM |
|---|---|
| SAE | Serioust 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 |
| VATS | Video-Assisted Thorascopic Surgery |
| WMA | World Medical Association |

# 1. INTRODUCTION

This Statistical Analysis Plan covers the statistical analysis and reporting for the protocol ON-1005 version 02 dated 25JAN2022. A detailed list of tables, listings and figures (TLFs) will be supplied in a separate, version-controlled document.

In case of discrepancies between the SAP and the protocol, the SAP will overrule the protocol with respect to analyses and reporting. Any deviations from the protocol will be documented at the end of this document.

# 2. STUDY OBJECTIVES

## 2.1 Objectives and Endpoints

The primary objective of this study is to investigate whether pegsitacianine can detect clinically significant events (CSEs) at the patient level, which 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 standard of care (SOC) biopsies

**Table 1. Objectives and Endpoints**

| **Objectives** | **Endpoints** |
|---|---|
| **Primary Objective** | **Primary Endpoint** |
| The primary objective of this study is to investigate whether pegsitacianine can detect clinically significant events (CSEs) at the patient level. | Rate of CSEs, which include:<br>1. The detection of disease that cannot be detected using standard procedures during the surgical resection of lung malignancies<br>2. The detection of synchronous lesions<br>3. The detection of positive surgical margins following primary tumor resection<br>4. Tumor-negative standard of care (SOC) biopsies |

| **Secondary Objectives** | **Secondary Endpoints** |
|---|---|
| Determine sensitivity, specificity, negative predictive values (NPPV), and positive predictive values (PPV) of the imaging agent at the level of the individual specimens, including lymph nodes. | Sensitivity, specificity. negative predictive values (NPV), and positive predictive values (PPV) at the specimen-level. |
| Evaluate safety and toxicity of pegsitacianine. | Incidence of TEAEs and SAEs, abnormalities in lab values, vital signs, or ECG results. |
| **Exploratory Objectives** | **Exploratory Endpoints** |
| Assess the how the SBR affects the diagnostic accuracy of pegsitacianine in detection of metastatic disease vs. normal tissue. | ROC analysis across various tumor-to-background ratio fluorescence thresholds. |
| Effect of camera system on diagnostic results. | Diagnostic results across various camera systems. |
| Assess amount of fluorescence shown in various types of specimens. | Mean fluorescence intensity and Specimen-to-background ratio |

## 3. STUDY DESCRIPTION

### 3.1 Study 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 [redacted] mg/kg dose of pegsitacianine administered [redacted] prior to standard of care (SOC) surgery. 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. [Note: Part 2 was not opened to enrollment.]

Patients will have a biopsy confirmed diagnosis, or a high clinical suspicion of a lung malignancy based on CT and/or PET imaging. A total of up to 40 patients will be enrolled in this trial. The sponsor reserves the right to perform an interim analysis following early enrollment (n ≤ 10 subjects) to determine the feasibility and benefit of pegsitacianine as an adjunct to SOC surgery.

The surgeon will attempt to perform their SOC resection of visible tumors. If the tumor is unable to be located under standard white light conditions, an appropriate near-infrared (NIR) imaging device may be used to aid in the location of 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 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, 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 or 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. Thus, each subject is their own ”intrapatient” control.

Collected surgical specimens will be documented as called out by the surgeon. The fluorescence status of each specimen will then be correlated to the final pathology for each and the performance metrics of pegsitacianine will be tabulated.

### 3.2 Study Treatment

Each patient will be administered a [redacted] mg/kg dose of pegsitacianine [redacted] The volume of drug and total dose will vary by patient and will depend on the patient's body weight.

## 4. SAMPLE SIZE AND POWER CALCULATION

The sample size for this study will be 40 subjects. This sample size is based on historical experience with the proposed analysis. A sample size of 40 subjects will be sufficient for meeting the endpoints described herein.

## 5. ANALYSIS ENDPOINTS

### 5.1 Efficacy Endpoints

#### 5.1.1 Clinically Significant Event Endpoints

Performance metrics will include the identification of a CSE detected by pegsitacianine. 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 (as synchronous disease), 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.

    - This occurs 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 that is found to be negative for tumor, and did not fluoresce during *ex situ* imaging.

A subject with any CSE will be considered a CSE responder.

### 5.1.2 Diagnostic Imaging Endpoints

Diagnostic performance of pegsitacianine will be assessed by calculating sensitivity, specificity, NPV, and PPV of pegsitacianine in detecting tumor containing tissue. The *ex situ* fluorescence status (any fluorescence vs. no fluorescence) of collected SOC and pegsitacianine guided specimens, as well as lymph nodes, will be compared to histological analyses of the collected specimens. Sensitivity and specificity for detecting tumor-containing tissue versus normal tissue

will be evaluated using tissue specimen fluorescence and the corresponding pathological outcomes.

True/false negative/positive definitions are as follows:

- False positive is defined as fluorescence was observed on the sample but the sample was not found to have tumor via pathology.
- False negative is defined as no fluorescence was observed on the sample but the sample was found to have tumor via pathology.
- True positive is defined as fluorescence was observed on the sample and the sample was found to have tumor via patholog1y
- True negative is defined as no fluorescence was observed on the sample and the sample was found not to have tumor via pathology.

Pegsitacianine sensitivity and PPV will be calculated using the following equations:

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

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

Pegsitacianine specificity values and NPV will be calculated using the following equations:

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

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

### 5.1.3 Fluorescence Endpoints

Specimen-to-background (SBR) 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.

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

Note: tumor-to-background (TBR) ratios are equivalent to SBR, but only relevant for specimens with tumors.

### 5.2 Safety Endpoints

- TEAE incidence, severity, and relationship to study drug
- Physical examination results
- Vital signs results
- ECG results
- Clinical laboratory values (hematology and serum chemistry)

## 6. ANALYSIS POPULATIONS

### 6.1 The Safety Population

All patients who receive at least one dose of pegsitacianine.

### 6.2 The Intent-to-Treat (ITT) Population

Identical to the Safety Population.

### 6.3 The Efficacy Population

All patients who receive >75% intended dose of pegsitacianine, had a minimum of one (1) image collected during their procedure and had the opportunity for post-SOC evaluation of the thoracic cavity.

## 7. ANALYTICAL PLAN AND STATISTICAL METHODS

### 7.1 General Considerations

All computations for statistical analyses will be performed using SAS® software, Version 9.4 or later. All SAS programs used in the production of statistical summary outputs will be validated with independent programming prior to finalization. In addition, all program outputs will be independently reviewed. The validation process will be used to confirm that all data manipulations and calculations were accurately done. Once validation is complete, a senior

statistical reviewer will perform a final review of the documents to ensure the accuracy and consistency with this plan and consistency within tables. Upon completion of validation and quality review procedures, all documentation will be collected and filed by the project statistician or designee.

If necessary, the statistical analytical plan and statistical methods section may be updated before the database lock. Any changes in statistical methods that may have an impact on the primary conclusions drawn from this clinical trial will be described in an amendment to the protocol. All other changes in the statistical plan will be described in the clinical study report (CSR). An explanation will be provided for deviations from the planned analysis.

### 7.2 Reporting Conventions

The following conventions will be applied to all data presentations and analyses:

- Continuous variables will generally be summarized by the number of patients with missing and non-missing values, mean, standard deviation, median, Q1 (quartile 1), Q3 (quartile 3), minimum, and maximum.
- Categorical variables will be summarized by the number and percentage of patients within each category. If not specified otherwise, the number of patients with non-missing values will be the denominator for percentage calculations. The number of patients with missing values will be presented.
- Confidence intervals will be reported as two sided at the 95% level.
- All mean and median values will be formatted to one more decimal place than the measured value.
- Standard deviation values will be formatted to two more decimal places than the measured value.
- Minimum and maximum values will be presented with the same number of decimal places as the measured value.
- The number and percent of responses will be presented in the form XX (XX %), where the percentage is in parentheses. Percentages will be rounded to the nearest percent. In

the case of a frequency of zero, the frequency and percentage will be presented as 0 rather than 0 (0%).

- Date variables will be formatted as DDMMMYYYY for presentation

All the analyses will be run displaying the results from Part 1. All rates will be summarized using counts and percentages.

### 7.3 Definition of Baseline

Baseline is defined as the measurement closest to, but prior to, the administration of study drug. No reassignment of visits will be conducted.

### 7.4 Handling of Dropouts and Missing, Unused and Spurious Data

All data will be analyzed.

No imputation of missing data will be performed, and analyses will use the observed cases only.

Formal outlier testing will not be performed. Data identified as potential outliers after review will be reviewed to determine whether they are spurious.

Spurious data are data determined to be incorrect upon review of site documents. If data is deemed to be spurious, the spurious data and the reason for it being spurious will be documented by the Sponsor in a Note-to-file. Data identified as spurious will be treated as missing unless they can be definitively corrected. If spurious data is identified, sensitivity analysis may be performed to assess the potential impact of any missing data or outliers on trial results by including the spurious data.

#### 7.4.1 Dates

Imputation of missing or partial dates is not expected, but if a complete date is required for calculations, the following algorithms will be applied:

- For the start date:
    - If year, month, and day are missing then use the minimum of the patient's first visit date or the consent date.
    - If either only month or month and day are missing then use January 1.

- If only day is missing, impute the first day of the month.

- For the end date:
  - If year, month, and day are missing then use the patient's last visit date.
  - If either only month or month and day are missing then use December 31.
  - If only day is missing then use the last day of the month.
  - Do not expand the record past the patient's last visit.
  - The original missing or partial date, the imputed complete date, and the indicator variable that indicates which dates were imputed will be retained in the database.

### 7.5 Adjustment for Multiplicity

There are no planned adjustments for multiple endpoints or analyses.

### 7.6 Adjustment for Multiple Centers

Differences between study centers will be not be incorporated into the statistical analyses for this study. There are no plans to analyze data within centers.

### 7.7 Patient Disposition

The number of screened patients will be summarized.

The number of enrolled patients will be summarized, along with the number and percentage of enrolled patients in all of the analysis populations, patients completing the study, patients withdrawing from the study and their primary reason for withdrawal, and the number of deaths, by Part and overall.

Data listings of patient disposition, screening failures, and patient assignment by Part will be created.

### 7.8 Protocol Deviations

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. Deviations which do have a potential impact on the study analyses will be considered "major".

The number and percentage of patients with at least one major protocol deviation and the categories of the major protocol deviations will be summarized for the Safety population.

All protocol deviations will be provided in a listing.

### 7.9 Patient Characteristics

Unless otherwise noted, all patient characteristics will use the Safety population.

#### 7.9.1 Baseline and Demographic Characteristics

All baseline characteristics will be summarized by each Part and overall for the Safety population. The following parameters will be summarized:

- Age (years)
- Race
- Ethnicity
- Sex
- Child-bearing Potential (if female)
- Height (cm)
- Weight (kg)
- BMI ($kg/m^2$)
- Karnofsky Performance Status

No hypothesis testing is planned for baseline characteristics, so the analysis will be purely descriptive.

Data listings of baseline characteristics and demographics including Karnofsky Status will be created.

### 7.9.2 Medical History

Medical history will be summarized for all non-cancer related medical events, previous cancers, and previous anti-cancer therapies. Medical conditions will be coded using Medical Dictionary of Regulatory Activities (MedDRA) 23.0 dictionary or higher and summarized by System Organ Class (SOC) and preferred term (PT) in the Safety population. A listing for the variables above will also be generated.

A summary and listing of previous alcohol, drug, and tobacco use will be provided.

### 7.9.3 Prior and Concomitant Medications and Procedures/Treatments

Prior and concomitant medication (as well as concomitant procedures/treatments) will be summarized by Part and overall in the Safety population. Medications will be coded with the World Health Organization (WHO) Drug Dictionary (B3 2021-09-01 DDE). Concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) Classification System level 2 and 4 codes (ATC2 and ATC4). Procedures/treatments will be coded using MedDRA 23.0 or higher and summarized by SOC and PT. A medication, procedure, or treatment will be considered to be concomitant if its end date is after the date of study drug administration. Medications with a missing end date will also be considered concomitant. A medication with an end date prior to the date of study drug administration will be considered prior medications.

Listings for prior and concomitant medications / procedures will be provided.

### 7.9.4 Administration and Exposure of Study Drug

Administration of study drug (planned dose level, total dose administered, total volume administered, infusion flow rate, infusion duration, subjects with infusion reactions, and subjects with dose interruptions) will be summarized using descriptive statistics. Infusion flow rate will be calculated as total volume administered÷infusion duration. A listing will also be generated.

### 7.9.5 Surgery Details and Specimen Collection

Surgery details including the following will be summarized on a patient level using descriptive statistics in the Safety population:

- Duration of surgery
- Location of tumor

- Method of tumor localization, including number of subjects with primary tumor(s) identified using NIR imaging
- Number of SOC specimens collected
- Number of additional lung nodules/areas found as a result of NIR imaging following SOC procedures and confirmed to be cancer
- Number of SOC specimens collected, found to be negative for tumor, and did not fluoresce during *ex situ* imaging
- Number of areas with fluorescence removed on:
    - Lung resection margins
    - Thoracic cavity
    - Lymph nodes
    - Total (any area)
- Number of areas with fluorescence on lung resection margins
- Subjects with fluorescence observed anywhere in thoracic cavity
- Number of lymph nodes collected as SOC surgery
- Time spent imaging
- Camera system used

A listing will also be generated.

### 7.10 Efficacy Endpoint Analyses

Imaging will be assessed on the Efficacy population.

### 7.10.1 Primary Analyses

#### 7.10.1.1 Analysis of Clinically Significant Events

The number and proportion of subjects with CSEs will be summarized by Part and overall. CSE responders will be summarized, as well as subjects with each type of CSE. These summaries will include 95% 2-sided confidence intervals for the proportion of CSEs calculated via Clopper-Pearson method.

A listing of all CSEs by patient will be provided.

### 7.10.2 Secondary Analyses

#### 7.10.2.1 Analysis of Diagnostic Imaging

The positive predictive value, negative predictive value, sensitivity, and specificity (specimen level) will be calculated and summarized for each Part and overall. The summary will include the true/false negative/positive rate and number of specimens. Two-sided 95% confidence intervals will be calculated for each rate via Clopper-Pearson method.

A listing by subject and specimen showing the specimen type (primary tumor/ additional SOC specimen, lung resection margin, thoracic cavity, lymph node), attainment by SOC or fluorescence (if primary/ additional SOC/ lymph), pathology result, fluorescence *in situ* and *ex situ,* and fluorescence measures (MFI and TBR) will be provided.

### 7.10.3 Exploratory Analyses

#### 7.10.3.1 Analysis of Fluorescence Intensity

A summary of MFI for the tumor (or suspected tumor), MFI for the normal background tissue, and TBR will be provided by Part and overall for each pathology result (tumor vs. no tumor). This summary will be at the specimen level, meaning each subject may have multiple specimens.

#### 7.10.3.2 ROC Analysis

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) and the 95% confidence interval of the 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.

#### 7.10.3.3 Camera System Analysis

An analysis on diagnostic results by camera system will be performed. 2x2 incidence tables comparing the true/false negative/positive results will be constructed for each camera system, and compared via the Breslow-Day test to assess if there is any effect of camera system on diagnostic results (e.g. to test if the common odds-ratio of each incidence table is equivalent across camera types). A summary including the diagnostic true/false negative/positive numbers and rates for each camera system, sensitivity, specificity, and p-value of this analysis will be provided.

#### 7.10.3.4 Other Exploratory Analyses

Relationships of sensitivity and specificity may be evaluated post-hoc with respect to additional covariates including, but not limited to, tumor size, tumor type, and tumor location.

### 7.11 Safety Endpoint Analyses

All the analyses in this section will be performed on the Safety population by Part and overall. Patients will be analyzed according to the actual treatment they received.

Repeated or unscheduled results will not be included in the summary statistics but will be included in the individual data listings.

### 7.11.1 Adverse Events

All adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). A TEAE is defined as an AE that occurs (or worsens) during or after study drug administration and through Day 21 after administration of study drug.

An overview of all AEs will be provided that will include:

- Number of AEs, TEAEs, non-serious TEAEs, and Serious Adverse Events (SAEs)
- Number of TEAEs and SAEs related to study drug
- Number of TEAEs related to study drug with CTCAE grade >= 3
- Number of AEs and study drug related SAEs resulting in:
    - Death
    - Subject discontinuation
    - Study drug discontinuation

TEAEs, treatment-related TEAEs, TEAEs by CTCAE grade, TEAEs that lead to discontinuation of study drug, SAEs, and SAEs that lead to discontinuation of study drug, will be summarized by system organ class (SOC) and preferred term (PT).

Infusion related reactions (IRR) will be summarized by PT, and a descriptive summary of the duration of IRR will be provided.

A listing of all AEs, including a separate listing for IRR, will also be generated.

### 7.11.2 Laboratory Data

The clinical laboratory data will be summarized by scheduled time point, Part, and change from baseline. Any laboratory data below/above the limit of quantification will be summarized using the value of the limit of quantification (upper or lower, respectively). A listing will be provided where 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 in the listing.

Repeated or unscheduled results will not be included in the by timepoint summaries but will be included when looking for extreme values and displayed in the individual data listings.

### 7.11.3 Vital Signs

Vital signs data will be summarized by time point, Part, and change from baseline. A listing will also be provided.

Repeated or unscheduled results will not be included in the summary statistics but will be included in the individual data listings.

### 7.11.4 Physical Exam

Physical examination results will be provided as a listing.

### 7.11.5 ECG

ECG results will be provided as a listing.

## 8. DEVIATIONS FROM ANALYSIS AS DESCRIBED IN THE PROTOCOL

No deviations from the protocol are planned.

## 9. APPENDIX 1: SCHEDULE OF EVENTS

| | Screening | Dosing | | Treatment | | |
|---|---|---|---|---|---|---|
| **Evaluation** | **Days –30 to –1** | **Day 0 Pre-Dose** | **Day 0 Post-Dose** | **Surgery ([redacted] – [redacted])** | **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 subjects, 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