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Pilot Study Evaluating Panitumumab-IRDye800 and ^{89}Zr-Panitumumab for Dual-Modality Imaging for Nodal Staging in Head and Neck Cancer

IRB-41878 **ENT0066** **NCT03733210** [redacted]

Relevant US FDA submissions:

^{89}Zr-panitumumab:	**IND 135573**, Andrei Iagaru, MD (IND-holder) (*NCI's IND 116229 is incorporated by cross-reference)*
Panitumumab-IRDye800:	IND 119474, incorporated by cross-reference (Eben L. Rosenthal, MD)
Cetuximab-IRDye800:	IND 115706, incorporated by cross-reference (Eben L. Rosenthal, MD)
IRDye800	Master File MF-25167, incorporated by cross-reference (LI-COR Biosciences, Inc)

SRC approval date: 03 April 2018

Version date	Document History
12 Jun 2017	Full draft, for initial IRB and pre-IND submission
20 Dec 2017	Initial Version – IRB/FDA/SRC
26 Mar 2018	Final SRC Comments
08 Jun 2018	Initial IND submission
07 Jul 2018	Received “Study may proceed” from FDA
04 Sep 2018	IRB approval granted for the study
01 Mar 2019	Protocol modifications: - Personnel update: Added Roan Raymundo as CRC, Kristen Cunanan as statistician (substituting previous statistician Vandana Sundaram), and Fred Baik as co-investigator - Clarify language regarding study procedures (section 9) - Addition Appendix C that includes a device list used for detection/imaging of ^{89}Zr-panitumumab
12 Apr 2019	Protocol modifications: - Removal of “Received an investigational drug within 30 days prior to first dose of panitumumab-IRDye800” as an exclusion criterion (section 3.2 and Appendix A). - Addition of correlative studies for primary tumor assessment (section 8). - Addition of collection of vital signs just before infusion of the ^{89}Zr-panitumumab bolus infusion (section 9.2.2) - Updated Appendix C with in-house developed 1mm resolution mobile PET system (collaboration with dr. Craig Levin, Radiology).
17 Sep 2019	Protocol modifications: - Extension of window of PET/CT from Day 0 to the Day before Surgery. - Inclusion of dosimetry sub-study (3-5 subjects) to collect dosimetry data that will provide insight on ^{89}Zr-panitumumab toxicity data. - Clarification on Day 15 and Day 30 study procedures. - Personnel updates: Removed Andrew Birkeland, MD as co-investigator. - Administrative changes to the protocol document.
13 Jan 2020	- Removal Stefania Chirita as CRC, addition of Grace Yi as CRC
20 Apr 2020	- Dose modification of panitumumab-IRDye800 Revisions to Appendix B include: - Addition of investigational device "OnLume Imaging System" - Removal of investigational device “PDE-NEO II”
8 May 2020	- Removes investigational device "OnLume Imaging System," erroneously identified as the intraoperative near-infrared fluorescence imaging device “Asimov 7685” - Adds investigational device “PDE-NEO II” back to protocol. - Add identifiers for IND number and NCT number
19 Aug 2021 (also in 6 Aug 2020 versions)	Protocol modifications (non-significant change) – Addition of investigational device: - “IMAGE1 S RUBINA System” for evaluation of excised tissue only, not for treatment decisions
19 Aug 2021 (also in 23 Apr 2021 / 5 April 2021 versions)	Protocol modifications: - Clarified expected Adverse Event collected window to only designated study dates - Cardiac Monitoring, Adverse Event and Serious Adverse Event reporting clarification - Change IND-holder to Andrei Iagaru, MD

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PROTOCOL SYNOPSIS

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<tr><td>TITLE</td><td>Pilot Study Evaluating Panitumumab-IRDye800 and ^{89}Zr-Panitumumab for Dual-Modality Imaging for Nodal Staging in Head and Neck Cancer</td></tr>
<tr><td>STUDY PHASE</td><td>Early phase 1 (pilot study)</td></tr>
<tr><td>INDICATION</td><td>Biopsy confirmed diagnosis of squamous cell carcinoma of the head and neck (HNSCC). Subjects diagnosed with any T stage, any subsite within the head and neck that are scheduled to undergo surgical resection. Subjects with recurrent disease or a new primary will be allowed.</td></tr>
<tr><td>INVESTIGATIONAL PRODUCT</td><td>^{89}Zr-panitumumab and panitumumab-IRDye800.
This study seeks to determine if the combined use of ^{89}Zr-panitumumab and panitumumab-IRDye800 can be used to identify tumor-loaded lymph nodes in subjects with HNSCC who undergo surgery with curative intent. The table below summarizes the planned dose level.
<table>
<tr><th colspan="2">Dosing Schedule</th></tr>
<tr><td>No of subjects</td><td>14</td></tr>
<tr><td>Dose of panitumumab-IRDye800</td><td>30 mg</td></tr>
<tr><td>Dose of ^{89}Zr-panitumumab</td><td>1.0 mCi, allowable range:
0.8 to 1.2 mCi (29 to 45 Mbq)</td></tr>
<tr><td>Specific Activity of ^{89}Zr-panitumumab</td><td>> 200 Ci/mmol</td></tr>
<tr><td>PET imaging post-injection</td><td>Day 0 to Day before Surgery</td></tr>
<tr><td>Timing of intraoperative imaging and surgical procedure post-injection</td><td>Day 2 to 5 post-infusion</td></tr>
</table></td></tr>
<tr><td>TREATMENT SCHEDULE</td><td>Subjects receive ^{18}F-FDG-PET/CT and/or ^{18}F-FDG-PET/MRI as part of their standard of care prior to study enrollment. After infusion of study agents on Day 0, subjects will undergo ^{89}Zr-panitumumab PET/CT imaging between Day 0 and the Day before Surgery. Subjects enrolled in the dosimetry sub-study will undergo imaging on day 0 to the day before surgery, for a total of 3 PET/CT scans.

Subjects will undergo surgical resection at 2-5 days after infusion (see figure below) based on expected half-life of panitumumab-IRdye800. Imaging will be performed intraoperatively using the optical imaging devices listed in Appendix B and the ^{89}Zr-detecting/imaging devices listed in Appendix C. The oncologic surgery will be conducted following the standard of care. *Ex vivo* imaging of the specimen prior to pathological assessment will be performed using non-invasive radioactive and fluorescence imaging modalities that do not violate or destroy the tissue.</td></tr>
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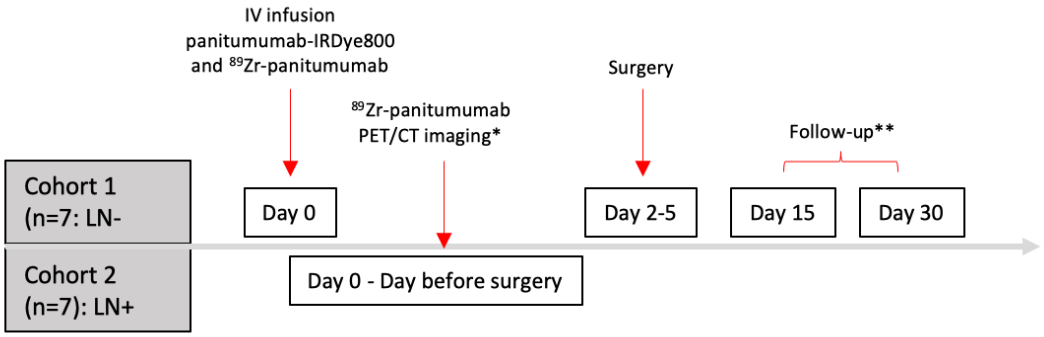
</table>

TREATMENT SCHEDULE	Subjects will undergo surgical resection at 2-5 days after infusion (see figure below) based on expected half-life of panitumumab-IRdye800. Imaging will be performed intraoperatively using the optical imaging devices listed in Appendix B and the ^{89}Zr-detecting/imaging devices listed in Appendix C. The oncologic surgery will be conducted following the standard of care. *Ex vivo* imaging of the specimen prior to pathological assessment will be performed using non-invasive radioactive and fluorescence imaging modalities that do not violate or destroy the tissue.  *Patients (n=3-5) in de dosimetry substudy will receive 3 PET/CT scans between day 0 and day of surgery. **On day 15 patients will come to the clinic. At day 30 patients will be called.
PRIMARY OBJECTIVE(S)	**Determine the sensitivity and specificity of ^{89}Zr-panitumumab for the detection of tumor-involved regional lymph nodes.** As defined by histological and/or pathological evaluation (gold standard), the total number of tumor-positive nodes will be determined and correlated to the radiologically-suspicious nodes identified by ^{89}Zr-panitumumab and ^{18}F-FDG contrast agents using PET/CT and/or PET/MRI imaging. The specificity and sensitivity of ^{89}Zr-panitumumab will be calculated and compared to the specificity and sensitivity of ^{18}F-FDG for metastatic lymph node identification.
SECONDARY OBJECTIVE(S)	**Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were <u>NOT</u> predicted tumor-positive by ^{89}Zr-panitumumab.** As defined by histological and/or pathological evaluation (gold standard), the total number of tumor-positive nodes will be determined and compared to the ^{89}Zr-panitumumab and ^{18}F-FDG-PET/CT and/or -PET/MRI imaging findings. We will compare the odds of a false negative using ^{89}Zr-panitumumab-PET relative to the odds of a false negative using a ^{18}F-FDG-PET and compare their respective negative predictive values.

EXPLORATORY OBJECTIVE(S)	• **Determine the sensitivity and specificity of panitumumab-IRDye800 for the detection of tumor-involved regional lymph nodes.** After isolation of lymph nodes from the specimen in the pathology lab, lymph node fluorescence will be measured prior to histological and/or pathological evaluation (gold standard). Sensitivity and specificity will be calculated by comparison of fluorescence intensity to histological and/or pathological evaluation (gold standard). Results will be compared to the sensitivity and specificity of ^{89}Zr-panitumumab and ^{18}F-FDG (primary objective). • **Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were <u>NOT</u> predicted tumor-positive by panitumumab-Irdye800.** We will compare the odds of a false negative using fluorescence imaging of panitumumab-IRDye800 to the odds of a false negative using ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET and compare the negative predictive values. Additionally, we will test the association of the panitumumab-Irdye800 results to ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET results with the Phi coefficient.
SAMPLE SIZE AND DURATION OF STUDY	It is anticipated that 14 subjects will be enrolled into the study. Safety data and adverse events will be reported at 15 days after the last subject receives the infusion, which is in excess of 4-fold the half-life of the largest dose of pantimumab-IRDye800 (2.3 days) and of the half-life of ^{89}Zr-panitumumab (3.3 days). Each subject will be followed for 30 days following the last dose of study medication. It is anticipated that the study will require 12 to 18 months to be completed.
STATISTICAL CONSIDERA-TIONS	<u>*General analysis tools*</u> All specimens from both cohorts will be pooled for the primary and secondary analyses. We will use the matched pair Durkalski's test that accounts for the correlation of multiple specimens per patent, to compare the specificity and sensitivity of ^{89}Zr-panitumumab-PET to ^{18}F-FDG-PET. We will compare the odds of a false negative with ^{89}Zr-panitumumab-PET relative to the odds of obtaining a false negative with ^{18}F-FDG-PET. <u>*Approach to drawing statistical inference*</u> Significance for all tests will be assessed at the 0.05 level and 95% confidence intervals will be reported and interpreted. <u>*Descriptive statistics*</u> We will present means and standard deviations (or medians and interquartile ranges when appropriate) for continuous characteristics such as maximum standard uptake values (SUV_{max}) as determined with ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET and radioactivity counts of ^{89}Zr-panitumumab in the node(s) as determined with the PET probe, and tumor-to-background ratios (TBRs) and mean florescence intensity (MFI) for panitumumab-Irdye800. For categorical variables such as drainage pattern and number of lymph nodes visualized, we will present frequency statistics. Graphical tools such as heat maps, histograms, and boxplots will be used to assess distributional properties of continuous variables. We will illustrate the nature of the relationship between SUV_{max}/radioactivity counts and/or MFI/TBRs and/or drainage pattern across all subjects, the relationship will be illustrated with a heat map and spaghetti plot. When appropriate, transformations – including a log-based transformation – may be considered to stabilize variance.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

^{89}Zr	Zirconium-89
AE	Adverse Event
BID	Twice Daily
BSA	Body Surface Area
CBC	Complete Blood Count
CI	Confidence Interval
CMAX	Maximum Concentration Of Drug
CNS	Central Nervous System
CRF	Case Report/Record Form
CTCAE	Common Terminology Criteria For Adverse Events
DLT	Dose-Limiting Toxicity
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
ECOG	The Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
FDA	Food and Drug Administration
GI	Gastrointestinal
GMP	Good Manufacturing Practice
Hgb	Hemoglobin
HNSCC	Head And Neck Squamous Cell Carcinoma
HTN	Hypertensions
ILD	Interstitial Lung Disease
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower Limit Of Normal
MTD	Maximum Tolerated Dose
NIR	Near Infra-Red
NHS	N-hydroxysuccinimide
PLT	Platelet
RBC	Red blood cell
SAE	Serious Adverse Event
SCC	Squamous Cell Carcinoma
TBR	Tumor to Background Ratio
SCCHN	Squamous cell carcinoma of the head and neck
TSH	Thyroid stimulating hormone
UAB	University of Alabama at Birmingham
ULN	Upper Limit Of Normal
UNK	Unknown
WBC	White blood cell
WHO	World Health Organization

SCHEMA

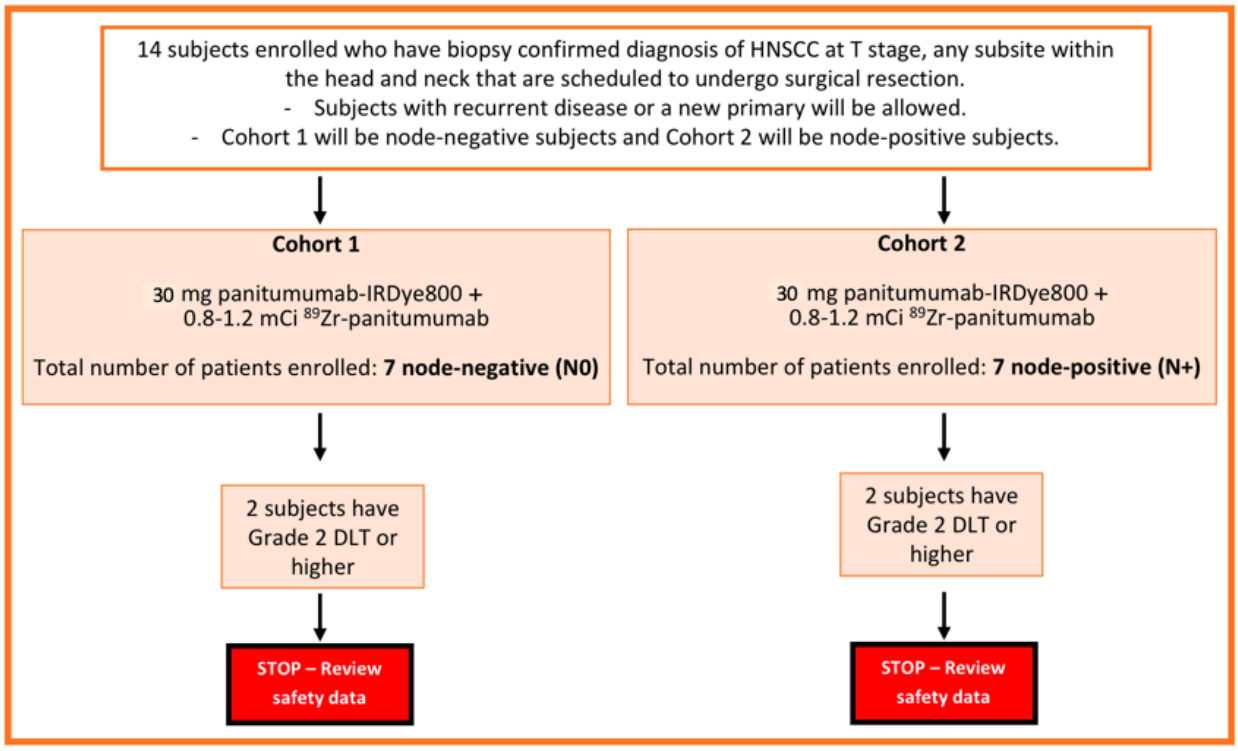


Figure 1. Study overview.

Study plan. The study is an open label, single institution, pilot study to determine the distribution pattern of ^{89}Zr-panitumumab and panitumumab-IRDye800 in subjects with head and neck squamous cell carcinoma (HNSCC) that undergo surgery with lymphadenectomy of curative intent. 30 mg of panitumumab-IRDye800 will be administered 1-5 days prior to surgery, intravenous (IV) over 15 min followed by an observation period of 30 min. ^{89}Zr-panitumumab will be systemically injected at a dose of 1.0 mCi +/- 20%, and with a specific activity greater than 200 Ci/mmol at the time of injection. The injected mass dose is less than or equal to 1 mg (7 nmol); the subject will be monitored for 1 hour post-administration. If at any point two subjects in a cohort experience a DLT, then the study will stop and safety data will be reviewed. The medical monitor and safety review will occur independently.

There will be 7 subjects enrolled into each cohort (total of 14 subjects in the study) whereby, based on the preoperative work-up, including a physical exam and evaluation of the pre-existing imaging data, subjects will be assigned to the node negative or node positive group.

Rationale for the two cohorts. To ensure the inclusion of sufficient subjects with clinically positive lymph nodes, we created a cohort structure. Including both node-positive and node-negative subjects will ensure that we will have tumor-positive nodes when performing the data analysis. Moreover, by analyzing data from node-positive subjects it will become possible to assess if, in case large lymph node metastasis are present, the tracer(s) can still reach the node or if rerouting (a phenomenon known to happen in sentinel node mapping, i.e., drainage to a “false” sentinel node occurs because the “true” sentinel node cannot be reached). As shown in the study schema, all subjects regardless if enrolled in cohort 1 or 2, will undergo the same treatment, and upon data collection statistical analysis will be performed with the pooled data.

1. OBJECTIVES

1.1. Primary Objective

Determine the sensitivity and specificity of ^{89}Zr-panitumumab for the detection of tumor-involved regional lymph nodes.

As defined by histological and/or pathological evaluation (gold standard), the total number of tumor-positive nodes will be determined for both ^{89}Zr-panitumumab and ^{18}F-FDG-PET imaging findings. The specificity and sensitivity of ^{89}Zr-panitumumab will be calculated and compared to the specificity and sensitivity of ^{18}F-FDG for metastatic lymph node identification. Additionally, we will determine the level of association between the ^{89}Zr-panitumumab-PET results and ^{18}F-FDG-PET results with the Phi coefficient.

1.2. Secondary Objective

Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were <u>NOT</u> predicted tumor-positive by ^{89}Zr-panitumumab labeling.

We will calculate the odds of a false negative using ^{89}Zr-panitumumab-PET relative to the odds of a false negative using a ^{18}F-FDG-PET and compare the negative predictive values of ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET.

1.3. Exploratory Objectives

- **Determine the sensitivity and specificity of panitumumab-IRDye800 for the detection of tumor-involved regional lymph nodes.**
  After isolation of lymph nodes from the specimen in the pathology lab, lymph node fluorescence will be measured prior to histological and/or pathological evaluation (gold standard). Sensitivity and specificity will be generated by comparison of fluorescence intensity and histological and/or pathological evaluation (gold standard). Findings will furthermore be compared to the sensitivity and specificity of ^{89}Zr-panitumumab and ^{18}F-FDG (primary objective).
- **Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were <u>NOT</u> predicted tumor-positive by panitumumab-Irdye800 labeling.**
  We will measure the level of association between panitumumab-IRDye800 results and ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET results with the Phi coefficient.
- **Dosimetry**
  In a subset of 3-5 subjects, dosimetry data will be collected to determine the toxicity of ^{89}Zr-panitumumab to the organs.

***NOTE**: It is planned that exploratory objectives will only be reported in publication(s), if any, but not on ClinicalTrials.gov.*

2. BACKGROUND

The most important determinant of survival for many cancer types is the presence of lymph node metastasis (1–3). In head and neck cancer, nodal metastases reduce 5-year survival to half, regardless of the primary tumor T stage (2–4). Likewise, regional metastases to axillary nodes in breast cancer decreases 5-year survival by approximately one-third (5). Although nodal disease can often be detected on routine physical exam or anatomic imaging with or without metabolic imaging, there are many cases where clinical and radiographic exams are negative, but microscopic disease is identified within the

primary nodal basin. This finding dramatically changes the diagnosis, and as a result, great effort is taken to harvest the first echelon lymph node(s) during removal of the primary tumor. When metastatic disease is too small to be detected preoperatively, this leads to down staging and under treatment.

Complete nodal dissection causes significant morbidity. The gold standard for detection of regional metastatic disease in HNSCC traditionally has been a regional nodal dissection that is associated with shoulder dysfunction and pain due to accessory nerve (cranial nerve XI) injury being common after neck dissection (6–9). Moreover, 60% to 80% of subjects undergoing a neck dissection with sectioning of the nerve have pain, limited abduction of the shoulder, and anatomic deformities such as scapular flaring, droop, and protraction (10). To reduce morbidity, there has been a gradual migration away from extensive dissection to more limited dissection offered by sentinel node biopsy where only the primary tumor draining lymph node(s) are removed for detailed histologic examination (5,11–14). Where sentinel node biopsy has already displaced regional lymphadenectomy in melanoma and breast surgery to avoid the associated deficits associated with this more aggressive approach, it is now also advocated for HNSCC (NCCN guidelines, version 1, 2017/2018).

Sentinel lymph node biopsy is performed as a surgical staging method to detect occult nodal disease in those tumor types with a high metastatic rate and where lymph node disease predicts outcome and informs adjuvant treatment decisions. The premise of sentinel node surgery is that regional metastasis from the primary tumor occurs through drainage of lymphatics to a single (or several) lymph nodes that can only be defined by mapping at the time of surgery. Sentinel node mapping occurs by local radiotracer or blue dye injection surrounding the primary tumor, thereby identifying the natural lymphatic drainage pattern from the primary tumor directly to the sentinel node(s). Sentinel nodes undergo aggressive histopathologic exam to assess for micro- or macro-metastatic disease.

Meta-analysis of sentinel node dissection in breast cancer, head and neck cancer, and melanoma have validated the accuracy of sentinel node biopsy in predicting occult lymph node metastasis (5,14–16), with a sensitivity of 91% (9) and a 3 to 10% false-negative rate. The false-negative rate can be due to poor injection technique or not anticipating the full extent of disease during injection, but the rerouting and skip metastasis phenomenon's have been postulated here as well (17–19). Moreover, although sentinel node mapping is superior to radiographic and clinical exam, it is still limited by the need for direct injection of the dye or radiolabeled compound. **Upstaging with sentinel node assessment can thus only be assessed in tumor types that are accessible for local injection.** This is significant because it influences standard of care (sentinel node surgery or complete lymphadenectomy) in these subject populations only. Identification of regional disease will inform prognostic information and/or determine the need and extent of adjuvant therapies for additional tumor types. Most importantly however, remains the fact that both lymphadenectomy and sentinel node biopsy do not allow for intraoperative lymph node staging. In both cases, post-operative (histo-) pathological evaluation is required to determine whether or not lymph node metastasis were present in the regional lymph node basin(s). Thus, HNSCC patients would benefit from an alternative technique that, ideally, allows for both preoperative as well as intraoperative lymph node staging.

Fluorescently labeled antibodies are currently used to target and visualize tumor cells intra-operatively. The safety and efficacy of cetuximab-IRDye800 were evaluated in the phase 1 clinical trial entitled "Open-label Study Evaluating the Safety and Pharmacokinetics of Escalating Doses of cetuximab-IRDye800 as an Optical Imaging Agent to Detect Cancer During Surgical Procedures," (incorporated to IND by cross-reference). A second phase 1 clinical trial is currently in progress, and is entitled "Open-label Study Evaluating the Safety and Pharmacokinetics of Escalating Doses of

Panitumumab-IRDye800 as an Optical Imaging Agent to Detect Head and Neck Cancer During Surgical Procedures" (IRB-35064, incorporated to IND by cross-reference).

This clinical trial is proposed to determine if a systemic injection of tracer labeled antibody will specifically accumulate in tumor containing lymph nodes to enhance identification. If successful, we will leverage existing technologies, PET and fluorescently labeled antibodies, in order to expand regional lymph node assessment to cancer types where direct injection is not possible.

2.1 Study Disease

This study is being performed in head and neck squamous cell carcinoma (HNSCC). Because the standard of care in this setting is currently complete lymphadenectomy, our proposed method will allow direct comparison with conventional lymphadenectomy and thus calculation of false positive and negative rates, sensitivity and specificity. Potential expansion to other cancer types not readily accessible for tracer injection, e.g., ovarian, uterine, and pancreatic cancer, are possible after showing the feasibility and validity of the approach in this study.

2.2 Study Agents

The study agents for this trial are **^{89}Zr-panitumumab** and **panitumumab-IRDye800.**

2.2.1 ^{89}Zr-panitumumab

^{89}Zr-panitumumab is a conjugate of ^{89}Zr, the radioactive isotope of zirconium (Zr), and panitumumab, the fully-humanized anti-epidermal growth factor receptor (anti-EGFR) IgG2 monoclonal antibody approved by FDA as Vectibix. The approved single agent indication for Vectibix is treatment of metastatic colorectal carcinoma with disease progression. See Section 5.1.1 for more information.

2.2.2 Panitumumab-IRDye800

Panitumumab-IRDye800 is a conjugate of panitumumab and IRDye® 800CW N-hydroxysuccinimide (NHS) ester infrared dye (LI-COR Biosciences, Inc., Lincoln, NE). See Section 5.1.2 for more information regarding panitumumab-IRDye800.

2.3 Rationale

Overall Impact of proposed studies on surgical oncology

Upstaging with sentinel node assessment has only been assessed in tumor where the tumors are accessible for local injection. We plan to determine if additional tumor types are amenable to regional lymph node staging after systemic injection of tracer labeled antibody. Identification of a novel lymph node detection methodology has the potential to 1) increase the sensitivity and decrease the false negative rate by avoiding the variability of direct injection; and 2) allow study of lymph nodes within non-superficial cancer types that are known to metastasize such as prostate, ovarian, uterine, lung and pancreatic cancers.

Although this study assesses an EGFR-directed antibody, this work, if successful, would have implications for any large protein-based imaging strategy. Currently, at least 12 such trials exist on ClinicalTrials.gov.

The methodology of this study and future studies could dramatically improve the standard of care in the following ways:

1) **Non-invasive detection of subclinical disease may allow improved subject selection for surgery as well as provide the surgeon with a roadmap for planning the surgical procedure.**
2) **Detect subclinical disease in the operating room at the time of resection:** Accurate identification of nodes may identify metastasis not detected by PET, and early identification of metastasis will improve survival.
3) **Real time feedback provided the radioactive and fluorescence signatures of the tracers:** Combined use could ultimately improve both the sensitivity and specificity of metastatic cancer detection and the ability to accurately resect it.
4) **Reduction of surgery time:** Although outside the scope of this study, successful identification of nodes in the clinic may reduce surgery time by reducing the need for complete nodal resection, reducing surgery time by up to 40 minutes. This might reduce the time subject is in surgery, and the cost of the surgery.
5) **Increased survival rate:** Replacing complete nodal resection as the standard of care with tumor-directed node resection might decrease the negative effects that accompany complete resection, and might also improve survival rate.
6) This is the first-in-human dual modality study involving ^{89}Zr-panitumumab and panitumumab-IRDye800 for regional lymph node staging specifically focusing on the identification of tumor-loaded lymph nodes. However, promising results might lead to expansion of this methodology to **use in other cancer types.** Intravenous injection of antibody-labeled radiotracers would allow regional lymph node staging in other cancer types where local injection is not possible. This would tremendously broaden the application to those indications for which lymph node staging is considered of great prognostic value (eg, gynecological, urological).

2.4 Study Design

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance:

- The primary purpose for the protocol is to develop a universal lymph node detection strategy. This study evaluates if systemic injections of ^{89}Zr-panitumumab in conjunction with pantitumumab-IRDye800 can be used to identify tumor-loaded lymph nodes to expand the capabilities of non-invasive and intraoperative cancer imaging.
- The interventional model is **Sequential**.
- There are **2 cohorts.** Both cohorts will receive the same treatment
- The study is **open-label**. No masking is used.
- The study is **non-randomized**.
- The primary outcome is **the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were predicted to be tumor-positive by ^{89}Zr-panitumumab PET/CT imaging.**
- The secondary outcome is **the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were NOT predicted tumor-positive by ^{89}Zr-panitumumab PET/CT imaging.**

The study is an open-label pilot study to determine the efficacy of ^{89}Zr-panitumumab in tumor-loaded node detection in subjects with HNSCC that undergo surgery with curative intent. It is anticipated that 14 subjects will be enrolled into the study. Subjects will be enrolled to the node negative (no suspicion of tumor-positive lymph nodes in the neck; cN0) or node positive (suspicion of tumor-positive lymph

nodes in the neck; cN+) group based on the preoperative work-up, which includes a physical exam and evaluation of the pre-existing imaging data.

All subjects will receive a dose of 30 mg panitumumab-IRDye800. Subjects will subsequently receive 1 mCi +/- 20% of ^{89}Zr-panitumumab. If at any point after infusion, 2 subjects experience a DLT (Grade 2 or greater toxicity), then the study will stop, and safety data will be reviewed.

The known rate of infusion reactions of panitumumab is 1% (package insert); therefore, we expect a DLT rate of approximately 1% or higher with panitumumab-IRDye800 or ^{89}Zr-panitumumab. The probability of observing two or more DLTs within a 10-subject cohort (requiring stopping the study) is 0.4%; if the DLT rate is as high as 5%, the probability of 2 or more DLT is 8.6%.

For an overview of the proposed study, see Study Schema (page 9), and the dosing schedule below. Briefly, subjects will undergo ^{18}F-FDG-PET prior to study enrollment, as is standard of care. Administration of panitumumab-IRDye800 will occur by IV over 15 minutes, followed by a 30-minute observation period. Administration of ^{89}Zr-panitumumab will occur by IV, per radioactive drugs administration policies. The subjects will be observed for 1 hour following the ^{89}Zr-panitumumab injection.

After infusion of study agents on Day 0, subjects will undergo ^{89}Zr-panitumumab PET/CT imaging. One scan will be performed at any time starting on Day 0 (after end of observation period post ^{89}Zr-panitumumab administration) and until the day before surgery (inclusive). Subjects enrolled in the dosimetry sub-study will undergo three scans, performed at any time starting on Day 0 (after end of observation period post ^{89}Zr-panitumumab administration) and until the day before surgery. If available, the scans will be performed on Day 0, Day 1 and on the day before surgery.

Subjects will undergo surgical resection 2-5 days after infusion of study drugs. Imaging will be performed intraoperatively using a novel beta probe and/or high-energy gamma probe (see Appendix C for a list of devices) and the optical imaging devices (see Appendix B for a list of devices), to assess the tumor and lymph node(s) prior to resection, and the wound bed after surgical removal. The surgery will be conducted in the standard of care, and the surgeon will not use the imaging data to make decisions.

Ex vivo, imaging of the specimens prior to pathological assessment will be performed using non-invasive imaging modalities that do not violate or destroy the tissue. These may include beta probe and/or high-energy gamma probe, an open-field imaging system, a closed-field imaging system and a fluorescence flatbed scanning device (see Appendix B and C for a list of devices). Additionally, a closed Cherenkov imaging system may be used to image the specimen after removal (see Appendix C). Methods have been described in detail in the literature (20–25).

Table 1: Dosing Schedule

No. of subjects	14
Dose of panitumumab-IRDye800	30 mg
Dose of ^{89}Zr-panitumumab	1.0 mCi, allowable range 0.8 to 1.2 (29 to 44 Mbq)
Specific Activity of ^{89}Zr-panitumumab	>200 Ci/mmol
PET imaging post-infusion*	Day 0 to the Day before Surgery
Timing of surgical procedure post-infusion	Day 2 to 5 post-infusion

Each subject will be followed for 30 days following the last dose of study medication.

Outcome measures to answer the primary and secondary objectives set:
Preoperative data:

1. Collect safety data on tracer-infusion.
2. The number and location of ^{18}F-FDG-PET positive nodes.
3. The number and location of ^{89}Zr-panitumumab-PET positive nodes.

Intraoperative data:

4. The number and location of ^{89}Zr-panitumumab-PET positive nodes.
5. The number and location of panitumumab-IRDye800 positive nodes.

Post-operative data:

6. Collect safety data in follow-up (up to 30 days post-infusion).
    a. This includes tests and observations to identify both acute and late-treatment specific toxicity: vital signs; physical exam; toxicity grading; assignment of performance status; hematology; and blood chemistries (basic metabolic profile to include magnesium and phosphorus). NCI Common Toxicity Criteria v5.0 will be used for grading of toxicity.
7. Determine *ex vivo* fluorescence imaging information on panitumumab-IRDye800 (method described previously in (21)).
8. Determine the histo-pathological status of the primary tumor.
9. Determine the histo-pathological status of the lymph nodes: tumor-positive (isolated tumor cells, micro- or macro-metastasis) or tumor-negative.
10. Determine, using immunohistochemistry, on the surgically excised tissue specimens:
    a. Tumor tissue viability
    b. EGFR expression
    c. Vessel density

    We have previously described the methods for analysis in (26).

<u>For qualification and quantification of the fluorescence signal and the radioactive signal, we follow the following rules:</u>

- *Intraoperative fluorescence assessment of panitumumab-IRDye800:*
  There will be no set level of fluorescence whereby we consider tissue positive or negative; it is based on operator's impression.
- *Ex vivo fluorescence assessment of panitumumab-IRDye800:*
  From the fluorescence images taken in the operation room, we will calculate tumor-to-background ratios (TBRs) as such to define a more quantitative read-out (similar to as described in the IRB-35064 study). Based on the current data available from our IRB-35064, in our 50 mg cohort, we found an average TBR of 6.53±1.2, a sensitivity of 89±5.1%, a specificity of 92±3.5% (AUC: 0.93±0.05). The NPV and PPV were 94±2.8% and 92±9.5%, respectively. We will extrapolate these findings to the current study to further validate the findings from our phase I study (IRB-35064).
- *Radioactive assessment of ^{89}Zr-panitumumab:*
  Following the standard of care evaluation of PET/CT scans, maximum standard uptake values (SUV_{max}) will be calculated for the accumulation of ^{89}Zr-panitumumab in tumor tissue and/or lymph nodes. As background reading, the SUV_{max} of the mediastinum will be determined.
  Tentative cutoff for 'imaging positive': an SUV_{max} value of the lymph nodes of minimally 1.4x the background (mediastinum). In all cases, (histo-) pathology serves as the gold standard for "true" positive / negative lymph nodes. Subsequently a ROC curve will be generated whereby we will select

the point of highest specificity and sensitivity and from there we will determine if our tentative cutoff was valid or if we need to adjust.

- *Comparison fluorescence and radioactivity-based findings:*
  All tissues that will be excised during surgery will be evaluated for the presence of radioactivity and fluorescence and scored as such being positive / negative. Evaluated tissues are either 1) fluorescent only; 2) radioactive only; 3) both radioactive and fluorescent; or 4) neither radioactive nor fluorescent. This will be done quantitatively (Y/N approach) and qualitatively (see above).
    - Because the antibody is the same, only the label (IRDye800 or 89-Zr) differs, we expect that tissue samples that are radioactive are also fluorescent and vice versa. However, this is something that will be investigated in this study.

Statistical considerations are described in detail in Section 11.

Dosimetry

Previously, for the antibody trastuzumab, it has been shown that when giving a pre-dose of cold (i.e. not radiolabeled) trastuzumab, prior to giving the radiopharmaceutical, the toxicity to the liver (and other organs) was found to be significantly lower resulting in the ability to go up on the dose of the radiopharmaceutical given, improving the preoperative image quality. For ^{89}Zr-panitumumab, there is little data on dosimetry. At the moment one publication reporting on data in 3 patients (28) whereby it was shown that upon giving a ~1 mCi dose, there's relative high toxicity to the organs, in particular the liver (28).

In our current study, in a subset of 3-5 subjects we will collect dosimetry data by assessing the biodistribution of ^{89}Zr-panitumumab over time as such to determine the toxicity of ^{89}Zr-panitumumab to the organs. To obtain dosimetry data, at various time points after injection of ^{89}Zr-panitumumab, PET/CT scans are to be acquired. The minimum number of PET/CT scans needed is three.

For the current study, these three PET/CT scans will be obtained between the end of observation period after the ^{89}Zr-panitumumab infusion on Day 0 and the day before surgery (inclusive). If available, the scans will be performed on Day 0, Day 1 and on the day before surgery.

For each patient, on the acquired PET/CT scans, volumes of interest (VOIs) will be drawn around the organs, tumor and lymph nodes. Subsequently, the total activity in each VOI will be recorded in units of Becquerel (Bq). Time activity curves (TACs) will be generated and then integrated to obtain the number of disintegrations (NDs) in each region. Hereafter the data will be loaded into dedicated software (e.g., OLINDA/EXM code (version 1.1.) after which the absorbed dose (in mGy/MBq) to each VOI can be determined. The internal dosimetry calculation model will be implemented following the Committee on Medical Internal Radiation Dose (MIRD) guidelines.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

1. Biopsy-confirmed diagnosis of squamous cell carcinoma of the head and neck.
2. Subjects diagnosed with any T stage, any subsite within the head and neck that are scheduled to undergo surgical resection. Subjects with recurrent disease or a new primary will be allowed.
3. Planned standard of care surgery with curative intent for squamous cell carcinoma.
4. Age ≥ 19 years.

5. Have acceptable hematologic status, coagulation status, kidney function, and liver function including the following clinical results:
   Hemoglobin ≥ 9 gm/dL
   White blood cell count > 3000/mm^3
   Platelet count ≥ 100,000/mm^3
   Serum creatinine ≤ 1.5 times upper reference range

3.2 Exclusion Criteria

1. Myocardial infarction (MI); cerebrovascular accident (CVA); uncontrolled congestive heart failure (CHF); significant liver disease; or unstable angina within 6 months prior to enrollment.
2. Previous bilateral neck dissection.
3. History of infusion reactions to monoclonal antibody therapies.
4. Pregnant or breastfeeding.
5. Magnesium or potassium lower than the normal institutional values.
6. Subjects receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents.
7. Subjects with a history or evidence of interstitial pneumonitis or pulmonary fibrosis.
8. Severe renal disease or anuria.
9. Known hypersensitivity to deferoxamine or any of its components.

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Enrollment Process

After signing an informed consent, subjects will be assigned a unique subject identification number based on the OnCore number and sequential numeric code (i.e. ENT0066-001, -002, etc.). The subject identification number will be used on all subject specific Case Report Forms (CRFs) and serious adverse event (SAE) forms. Participant information should be entered into OnCore within 5 business days.

3.5 Study Timeline

Primary Completion:

The study will reach primary completion 24 months from the time the study opens to accrual.

Study Completion:

The study will reach study completion 36 months from the time the study opens to accrual.
The anticipated study start date: July 2018
The anticipated study completion date: October 2020

4. TREATMENT PLAN

4.1 Treatment Schedule

This study will assess a microdose of ^{89}Zr-panitumumab and an imaging dose of panitumumab-IRDye800 in 14 subjects. The study drugs will be administered on Day 0, and subjects will undergo one ^{89}Zr-panitumumab PET/CT imaging anytime starting on Day 0 and the Day before Surgery (inclusive).

Subjects enrolled in the dosimetry sub-study will undergo imaging anytime starting on Day 0 and until the day before surgery (inclusive), for a total of three scans; if available, the scans will be performed on Day 0, Day 1 and on the day before surgery.

Subjects will undergo surgical resection at 2 to 5 days after infusion of study drug (see Table 1). Intraoperative and post-operative imaging data will be collected to answer the study objectives. The study will be stopped if any 2 subjects experience a DLT of grade 2 or higher that is likely to be related to the study drugs administered.

4.2 Administration of study agents

Panitumumab-IRDye800

Administration of panitumumab-IRDye800 will be performed consistent with institutional policy. The solution will be diluted to a total volume of 100 mL with 0.9% sodium chloride injection, USP. Dosage level will be 30 mg panitumumab-IRDye800 (< 1/12 of a therapeutic dose of panitumumab). Administration of panitumumab-IRDye800 will occur by IV over 15 minutes followed by a 30-minute observation period. Additional treatment for infusion reactions and electrolyte imbalances will be given as per institutional policy, if needed.

^{89}Zr-panitumumab

After infusion of the panitumumab-IRDye800 the subject will receive the prescribed injection of ^{89}Zr-panitumumab. Approximately 75 MBq (2 mCi) of ^{89}Zr will be conjugated with panitumumab antibody. The dosage will be <1 mg ^{89}Zr-panitumumab (< 1% of a therapeutic dose of panitumumab). The ^{89}Zr-panitumumab will be systemically injected via IV with a dose of 1 mCi (+/- 20%). Subjects will be observed for 1 hour after administration. Additional treatment for infusion reactions and electrolyte imbalances will be given as per institutional policy, if needed. Staff will be trained to handle the amounts of radioactive material represented by ^{89}Zr-panitumumab. The appropriate equipment will be used to administer and transport the ^{89}Zr-panitumumab.

A low-dose CT will be obtained from vertex to toes; this will use 120 kV and dose modulation based on body habitus, ranging 10-105 mA. This will be followed by a static PET emission scan over the same area.

All infusions/injections will be done according to Stanford facilities guidelines. Consistent with the panitumumab package insert, the study drugs ^{89}Zr-panitumumab and/or panitumumab-IRDye800 will not be mixed with, or administered as an infusion with other medicinal products, nor infused through a peripheral intravenous line or indwelling intravenous catheter.

4.3 Dose Rationale

Rationale for dose of 1.0 mCi (37 Mbq) of ^{89}Zr-panitumumab and PET imaging at Day 1 to 2 post-infusion. The absorbed dose to the liver is relatively high, which limits the dose that can be administered to humans to approximately 1.0 mCi. The human studies reported in the literature have used 1 or 2 mCi and achieved good image quality (27,28).

It is hypothesized that the systemic circulation of the antibody-drug will accumulate specifically within the tumor during the first 24 to 36 hours after infusion based on our preliminary data previously presented. However, we do expect that longer periods of accumulation in the primary tumor (2 to 5 days) will result in non-specific drainage of dye into the regional lymph nodes from non-specific lymphatic drainage form the tumor.

Rationale for dose of 30 mg panitumumab-IRDye800 without loading dose. Previously we identified a flat dose of 50 mg of panitumumab-IRDye800 as the optimal dose for intraoperative imaging in HNSCC, but we have recently reviewed data for almost 54 patients who received panitumumab-IRDye800 and have evidence to support lowering the dose to 30 mg:

1) We have shown that the tumor-to-background (TBR) ratio is the same for doses in the range 25-50 mg. As can be seen in Figure 2 and Table 2, irrespective of the dose given, a fixed dose or a dose in mg/kg, the varies between 2-3. A dose of 30 mg would translate to a dose of approximately 0.41 mg/kg (assuming a patient weighing 73 kg). At this dose, we also do not expect that the timing is going to be of an issue, something which we did see when giving a lower dose of around 0.3 mg/kg (Figure 3).
2) There has been progressive improvement in our understanding of the benefit of the surgical imaging over the past 2 years, and we are now looking at margins in the primary specimen, rather than microscopic disease (29–31);
3) In the lymph node data that we recently analyzed, we found a high sensitivity and specificity (84.6% and 94.0%, respectively) for identifying metastatic nodes. Furthermore, we demonstrated that a lower dose reduced the number of false-positives lymph nodes identified. Combined, this data leads us to believe that a lower dose (<0.5 mg/kg) may thus allow for improved metastatic lymph node detection compared to a >0.5 mg/kg dose of panitumumab-IRDye800 (32) (Figure 4);
4) The imaging software that we have been using continues to improve, and with this increased sensitivity, allows us to detect lower amounts of drug (both LICOR Biosciences Inc., and Novadaq/Stryker are continuously working on improved imaging devices – we have long-standing collaboration agreements in place);
5) As of June 2019 we received our second lot of panitumumab-IRDye800 and we have seen an improved fluorescence signal coming from this lot compared to the previous batch.

With that in mind, we would like to change the dose to 30 mg.

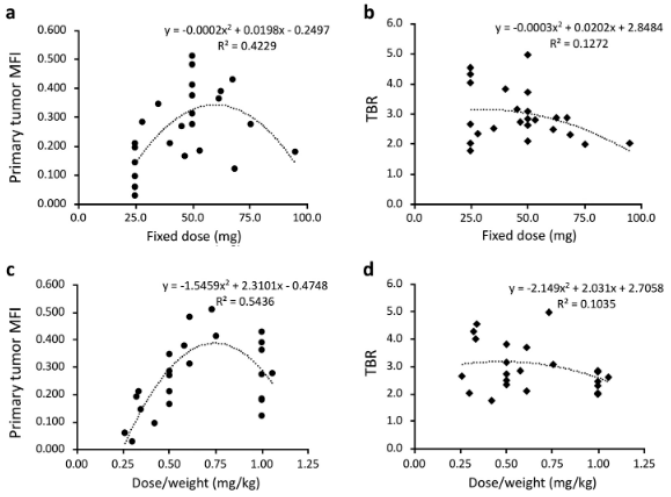


Figure 2. a) Correlation between primary tumor mean fluorescence intensity (MFI) and the fixed-dose of panitumumab-IRDye800 administered to the patient; b) Correlation between tumor-to-background ratio (TBR) and the fixed-dose of panitumumab-IRDye800 administered to the patient; c) Correlation between primary tumor MFI and the average dose of panitumumab-IRDye800 administered to the patient; d) Correlation

Table 2. Comparison of clinical variables for primary tumor MFI and TBRs.

Variable		MFI		TBR	
		Mean	*p* value	Mean	*p* value
Dose	High	0.33	**	2.8	N.S.
	Low	0.18		3.1	
Time of infusion-surgery	≤2 days	0.30	*	3.1	N.S.
	>3 days	0.18		2.5	
Unlabeled dose	100 mg	0.27	N.S.	2.7	N.S.
	0 mg	0.26		3.2	
Age	≤60 years	0.25	N.S.	3.0	N.S.
	>60 years	0.27		2.9	
Gender	Male	0.24	N.S.	3.0	N.S.
	Female	0.30		2.9	
Primary site	Tongue	0.32	N.S.	3.6	*
	Other	0.23		2.6	
Tumor size	≤40 mm	0.27	N.S.	3.0	N.S.
	>40 mm	0.25		2.8	

*p<0.05; **p<0.01; N.S. = not significant
MFI = mean fluorescence intensity; TBR = tumor-to-background ratio. *Adapted from Nishio et al., MIB 2018.*

between TBR and the average dose of panitumumab-IRDye800 administered to the patient. *Adapted from Nishio et al., MIB 2018.*

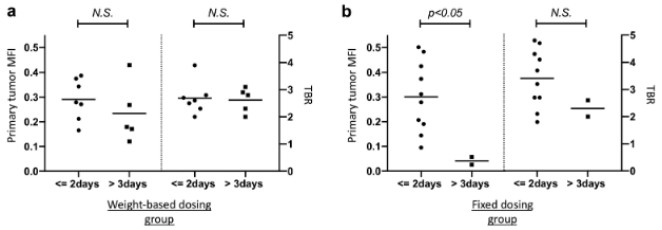


Figure 3. a) Within the weight-based dosing group, no significant difference between primary tumor MFI and TBR was found when looking at the time between infusion and surgery (1-2 days vs. >3 days); b) In the fixed-dosing group, significantly higher primary tumor MFIs were found when the infusion to surgery window was 1-2 days vs. 3 days (p<0.05). *Adapted from Nishio et al., MIB 2018.*

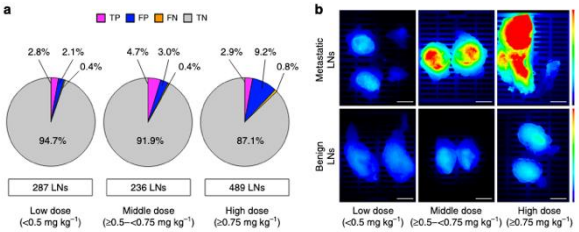


Figure 4. Effect of the panitumumab-IRDye800 on the find rate of false-positive lymph nodes. a) Rates of true positive, false positive, false negative and true negative lymph nodes (LNs) over three groups by weight adjusted dose. b) Representative fluorescence images of metastatic and benign lymph nodes for the three dose groups.
TP = true positive (fluorescence positive, histopathologically confirmed metastatic LN); TN = true negative(fluorescence negative, histopathologically benign LN);FP= false positive (fluorescence positive, histopathologically benign LN);FN = false negative(fluorescence negative, histopathologically confirmed metastatic LN).

Surgical timing 2-5 days post-infusion. Calculating the TBR over time can be used to identify the optimal day of surgery that will provide the greatest tumor contrast. The proposed time course for imaging and surgery is based on data obtained from our cetuximab-IRDye800 and panitumumab-IRDye800 study, which has demonstrated increasing fluorescence intensity up to 3 days post injection of the antibody-dye conjugate (36). We found that most of our subjects had accessible tumors that could be imaged non-invasively in the clinic over time since oral cavity lesions can be well-visualized as well as cutaneous manifestations. The optimal imaging time point for ^{89}Zr-panitumumab is nearly identical to that of panitumumab-IRDye800. Thus, we plan to perform the surgery or image for up to 5 days to identify the optimal TBR as shown in our previous study.

Rationale for observation period. We will follow up with subjects on Day 15, which is greater than 4 half-lives of panitumumab. The half-life of panitumumab when given in multiple doses is known to be approximately 7 days (per package insert). However, when administered at smaller doses the half-life is less since the pharmacokinetics are non-linear. We know that at 2 mg/kg, the half-life is 2.3 days (FDA Drug Approval Package, Clinical Pharmacology Biopharmaceutics Review, application 125147), which is less than the 1 mg we are administering as our highest dose. Because the highest proposed dose in the current study is 50 mg of panitumumab-IRDye800, it is not expected to be detectable beyond 12 days after administration in our subject population. Fifteen days is also greater than 4-fold the half-life of ^{89}Zr, which is 78.4 hours (3.3 days). The duration of 15 days after infusion is therefore chosen as the time point to assess for safety. The study will also follow up with subjects on Day 30.

Pharmacokinetic intervals. Because the pharmacokinetics of single dose of panitumumab are well known, only limited pharmacokinetics will be performed. Blood levels will be monitored on Day 0 before infusion; 1 to 2 hours after infusion; and on the day of surgery, and on Day 15 for panitumumab-IRDye800 and free IRDye800. Clearance of panitumumab occurs through the binding in the liver and through the reticuloendothelial system as occurs with endogenous immunoglobulin.

4.4 Definition of Dose-limiting toxicity (DLT) and Cohort Expansion

Adverse events will be graded according to the CTCAE v5.0 (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

Dose-limiting toxicity will be defined as any Grade 2 or greater toxicity that is determined by the principal investigator to be possibly, probably or definitely-related to study drugs and clinically significant for subjects who received panitumumab-IRDye800 and ^{89}Zr-panitumumab for this study. Clinically significant is defined based on NCI definition – a result that is large enough to affect a subject's disease state in a manner that is noticeable to the subject and/or caregiver or requires intervention.

4.5 Study Drug Discontinuation

Study drug will be discontinued for any of the following:

- Subject withdraws consent for the study
- Death attributed to study drug
- Serious systemic anaphylactic reaction (Grade 3 or higher)
- Any serious rare reactions attributed to study drug

If one of these events occurs, and is a serious adverse event (see Section 7.2), the FDA, IRB and Stanford DSMC will be notified immediately and there will be a comprehensive review of the safety data prior to resuming and further study drug administration.

4.6 General Concomitant Medication and Supportive Care Guidelines

Other than chemotherapy (including any EGFR-targeting agent), subjects may continue any medication they are receiving at study entry for underlying medical conditions. All medications taken at time of study entry will be recorded. Any changes in concomitant medication, including additions, discontinuations, and dose changes occurring during the study will be recorded.

Medications administered on the Day 0 until Day 30 will not be collected unless given for a reportable adverse event or reportable serious adverse event (see Section 7 for definitions).

4.7 Criteria for Removal from Study

Treatment will be stopped and subjects will be removed from study due to unacceptable adverse events, withdrawal of consent or non-compliance.

4.8 Alternatives and Risk Protection / Mitigation

Alternatives

- This study is in the setting of tumor excision of biopsy-confirmed diagnosis of HNSCC, and the experimental component is infusion of ^{89}Zr-panitumumab and panitumumab-IRDye800, which may help visualize the tumor and tumor margins as well as identify tumor-loaded lymph nodes for the surgeon. The surgery itself is considered regular medical care. Therefore, the alternative to participating in this study is to have the tumor excision surgery without the ^{89}Zr-panitumumab and panitumumab-IRDye800 infusion.

Procedures to protect against and minimize potential risks:

- This study is designed enroll appropriate subjects populations (see Section 3.1 Inclusion Criteria, and Section 3.2 Exclusion Criteria).

- Subjects will be monitored regularly by the investigators and the study team after investigational ^{89}Zr-panitumumab and panitumumab-IRDye800 and surgery, through 30 days (± 1 week) post-^{89}Zr-panitumumab and panitumumab-IRDye800 treatment, for adverse events by means of physical exams, blood tests, and ECGs.
- Subjects will be given a 24-hour emergency number in case problems arise between clinic visits.
- Subject records will be kept in a secure location at Stanford University Medical Center accessible only to authorized personnel.

5 INVESTIGATIONAL AGENT INFORMATION

5.1 Description of Study Drugs

This IND proposes a study of the sequential use of 2 investigational imaging agents, ^{89}Zr-panitumumab and panitumumab-IRDye800.

9.2.3. 5.1.1 ^{89}Zr-panitumumab

Zirconium is a Group IVB transition metal, and its isotope, ^{89}Zr, is an excellent candidate for ImmunoPET. ^{89}Zr decays through positron emission and electron capture with a half-life of 78.4 hours (3.3 days), which is similar to the optimal TBR time for most monoclonal antibodies. An advantage of ^{89}Zr is that it requires less energy to produce. One limitation of this isotope is that its high gamma emission of 908.97 keV restricts the administered dose in subjects.

Over the years, many preclinical studies have been performed *in vivo* with various ^{89}Zr-labeled antibodies. At present, there are 48 studies listed on ClinicalTrials.gov: 9 with ^{89}Zr-labeled bevacizumab, 8 with ^{89}Zr-labeled trastuzumab, 4 with ^{89}Zr-labeled cetuximab, and 2 with ^{89}Zr-labeled panitumumab. The latter two studies are completed and were performed by NCI.

Of the clinicaltrials.gov published studies, 3 report on the use of DFO (deferoxamine) that is proposed to be used to link ^{89}Zr to panitumumab for this study.

^{89}Zr-panitumumab is an established investigational PET agent by the FDA. Peer-reviewed published dosimetry data and first human experience with ^{89}Zr-panitumumab does not report any major adverse events related to ^{89}Zr-panitumumab (27-28). In Lindenberg et al (28), three subjects with metastatic colon cancer were administered approximately 1 mCi (37 MBq) of ^{89}Zr-panitumumab by IV, with whole body (base of skull to mid-thigh) static images obtained at 2-6 hours, 1-3 days, and 5-7 days post-injection. One participant only completed two post-injection scans due to scheduling constraints; the other two participants completed three post-injection scans. Based on whole organ image contours of the liver, kidneys, spleen, stomach, lungs, bone, gut, heart bladder and psoas muscle, the whole-body effective dose was estimated between 0.264 mSv/MBq (0.97 rem/mCi) and 0.330 mSv/MBq (1.22 rem/mCi).

The following dosimetry tables are reproduced from Lindenberg et al. (28).

Table 3: Results from OLINDA 1.1 estimating the dose using the residence times for the two subjects who were scanned three times

Target Organ	Alpha	Beta	Photon	Total	EDE Cont.	ED Cont.
Adrenals	0.00E000	9.13E-03	3.68E-01	3.77E-01	2.26E-02	1.88E-03
Brain	0.00E000	9.13E-03	3.77E-02	4.68E-02	0.00E000	2.34E-04
Breasts	0.00E000	9.13E-03	9.44E-02	1.04E-01	1.55E-02	5.18E-03
Gallbladder Wall	0.00E000	9.13E-03	6.06E-01	6.15E-01	3.69E-02	0.00E000
LLI Wall	0.00E000	8.63E-02	1.46E-01	2.32E-01	0.00E000	2.79E-02
Small Intestine	0.00E000	1.07E-01	2.18E-01	3.25E-01	0.00E000	1.62E-03
Stomach Wall	0.00E000	3.19E-02	2.25E-01	2.57E-01	0.00E000	3.08E-02
ULI Wall	0.00E000	6.83E-02	2.91E-01	3.59E-01	0.00E000	1.80E-03
Heart Wall	0.00E000	7.20E-02	2.60E-01	3.32E-01	0.00E000	0.00E000
Kidneys	0.00E000	1.46E-01	4.04E-01	5.50E-01	3.30E-02	2.75E-03
Liver	0.00E000	6.27E-01	1.31E000	1.94E000	1.16E-01	9.69E-02
Lungs	0.00E000	3.20E-02	1.93E-01	2.25E-01	2.70E-02	2.70E-02
Muscle	0.00E000	9.13E-03	1.10E-01	1.19E-01	0.00E000	5.97E-04
Ovaries	0.00E000	9.13E-03	1.51E-01	1.60E-01	3.99E-02	3.19E-02
Pancreas	0.00E000	9.13E-03	3.62E-01	3.71E-01	0.00E000	1.86E-03
Red Marrow	0.00E000	6.67E-03	1.30E-01	1.37E-01	1.64E-02	1.64E-02
Osteogenic Cells	0.00E000	2.33E-02	1.03E-01	1.26E-01	3.79E-03	1.26E-03
Skin	0.00E000	9.13E-03	6.67E-02	7.58E-02	0.00E000	7.58E-04
Spleen	0.00E000	5.44E-01	6.71E-01	1.21E000	7.29E-02	6.07E-03
Testes	0.00E000	9.13E-03	5.00E-02	5.91E-02	0.00E000	0.00E000
Thymus	0.00E000	9.13E-03	1.03E-01	1.12E-01	0.00E000	5.59E-04
Thyroid	0.00E000	9.13E-03	5.66E-02	6.58E-02	1.97E-03	3.29E-03
Urinary Bladder Wall	0.00E000	9.13E-03	8.49E-02	9.40E-02	0.00E000	4.70E-03
Uterus	0.00E000	9.13E-03	1.29E-01	1.38E-01	0.00E000	6.88E-04
Total Body	0.00E000	2.94E-02	1.44E-01	1.73E-01	0.00E000	0.00E000
Effective Dose Equivalent (mSv/MBq)			3.86E-01			
Effective Dose (mSv/MBq)			2.64E-01			

Table 4: Results from OLINDA 1.1 estimating the dose using all three subjects but only the first two scans to generate the time activity curve from which average residence times were calculated

Target Organ	Alpha	Beta	Photon	Total	EDE Cont.	ED Cont.
Adrenals	0.00E000	1.24E-02	4.67E-01	4.79E-01	2.87E-02	2.40E-03
Brain	0.00E000	1.24E-02	5.11E-02	6.35E-02	0.00E000	3.18E-04
Breasts	0.00E000	1.24E-02	1.22E-01	1.35E-01	2.02E-02	6.75E-03
Gallbladder Wall	0.00E000	1.24E-02	7.74E-01	7.86E-01	4.72E-02	0.00E000
LLI Wall	0.00E000	9.90E-02	1.78E-01	2.77E-01	0.00E000	3.32E-02
Small Intestine	0.00E000	1.23E-01	2.69E-01	3.92E-01	0.00E000	1.96E-03
Stomach Wall	0.00E000	3.62E-02	2.67E-01	3.04E-01	0.00E000	3.64E-02
ULI Wall	0.00E000	7.98E-02	3.58E-01	4.38E-01	0.00E000	2.19E-03
Heart Wall	0.00E000	1.06E-01	3.43E-01	4.49E-01	0.00E000	0.00E000
Kidneys	0.00E000	2.18E-01	5.30E-01	7.49E-01	4.49E-02	3.74E-03
Liver	0.00E000	8.02E-01	1.68E000	2.48E000	1.49E-01	1.24E-01
Lungs	0.00E000	4.17E-02	2.47E-01	2.89E-01	3.47E-02	3.47E-02
Muscle	0.00E000	1.24E-02	1.41E-01	1.53E-01	0.00E000	7.67E-04
Ovaries	0.00E000	1.24E-02	1.87E-01	1.99E-01	4.99E-02	3.99E-02
Pancreas	0.00E000	1.24E-02	4.37E-01	4.49E-01	0.00E000	2.25E-03
Red Marrow	0.00E000	9.09E-03	1.66E-01	1.76E-01	2.11E-02	2.11E-02
Osteogenic Cells	0.00E000	3.18E-02	1.34E-01	1.66E-01	4.97E-03	1.66E-03
Skin	0.00E000	1.24E-02	8.62E-02	9.87E-02	0.00E000	9.87E-04
Spleen	0.00E000	4.36E-01	5.97E-01	1.03E000	6.20E-02	5.16E-03
Testes	0.00E000	1.24E-02	6.66E-02	7.90E-02	0.00E000	0.00E000
Thymus	0.00E000	1.24E-02	1.36E-01	1.48E-01	0.00E000	7.40E-04
Thyroid	0.00E000	1.24E-02	7.59E-02	8.84E-02	2.65E-03	4.42E-03
Urinary Bladder Wall	0.00E000	1.24E-02	1.10E-01	1.23E-01	0.00E000	6.13E-03
Uterus	0.00E000	1.24E-02	1.62E-01	1.74E-01	0.00E000	8.71E-04
Total Body	0.00E000	3.77E-02	1.83E-01	2.21E-01	0.00E000	0.00E000
Effective Dose Equivalent (mSv/MBq)			4.65E-01			
Effective Dose (mSv/MBq)			3.30E-01			

^{89}Zr-panitumumab will be manufactured at the MIPS Cyclotron and Radiochemistry Facility per the manufacturing information in [redacted] (IND-holder Andrei Iagaru, MD).

Following the ^{89}Zr-panitumumab bolus injection, a PET/CT the scan will be performed anytime starting on Day 0 until the day before surgery (inclusive). The whole body effective dose for this procedure is the 3-5 mSv. In the subjects that will participate in the dosimetry sub-study whereby they will receive a total of three PET/CT scans, the whole body effective dose of the PET/CT scans will be 9-15 mSv.

9.2.4. 5.1.2 Panitumumab-IRDye800

Panitumumab-IRDye800 was manufactured from commercially-sourced panitumumab and cGMP IRDye® 800CW N-hydroxysuccinimide (NHS) ester infrared dye (LI-COR Biosciences, Inc.) at the GMP biologics facility at LI-COR Biosciences.

Panitumumab is as described above. IRDye800CW has a molecular weight of 1166 Da and is supplied as a lyophilized powder. It can be dissolved in water or DMSO at concentrations up to 20 mg/mL. The dye

in 1:1 solution of phosphate buffered saline (PBS):methanol has an absorbance maximum of 777 nm and an emission maximum of 791 nm. IRDye800CW is a near-infrared fluorescent imaging dye that can be easily bound to proteins through its NHS Ester group. IRDye800CW is not FDA-approved, and much of the information regarding IRDye800 remains proprietary. However, LI-COR Biosciences manufactures IRDye800 under cGMP conditions, and has submitted a drug master file (MF) to the FDA (MF-25167) that is cross-referenced for the IND under which this study is conducted.

Panitumumab-IRDye800 is produced by mixing the panitumumab and IRDye800 components in phosphate buffer at pH 8.5 and allowing the conjugation reaction to proceed at 20 to 25ºC. The conjugation reaction is followed by a buffer exchange into pH 7.4 PBS. The desired stoichiometry for the product is approximately 1.5 dye molecules per antibody molecule.

Panitumumab-IRDye800 has been previously evaluated as an imaging agent in several Stanford studies, in patients with head and neck cancer (IRB-35064), brain tumors (IRB-43179), lung cancer (IRB-43102), and pancreatic cancer (IRB-42237) for a total of 77 patients studied to date. A related agent, cetuximab-IRDye800, has been used in Stanford study IRB-35068, IRB-35789 and IRB-37595.

5.2 Expected Toxicities

9.2.5. 5.2.1 Expected Toxicities of ^{89}Zr-panitumumab

The toxicities of ^{89}Zr-panitumumab are analogous to the known toxicities of panitumumab (see package insert); panitumumab-IRDye800; and ^{18}F-FDG-PET with the additional toxicities of the IRDye800 chelator, deferoxamine. The toxicities of deferoxamine as elaborated in the prescribing information are reviewed below.

5.2.1.1 Deferoxamine Toxicities

Deferoxamine: Deferoxamine (Desferal) is approved throughout much of the world for the treatment of acute iron intoxication and of chronic iron overload due to transfusion-dependent anemias. Deferoxamine chelates iron by forming a stable complex that prevents the iron from entering into further chemical reactions. It readily chelates iron from ferritin and hemosiderin but not readily from transferrin; it does not combine with the iron from cytochromes and hemoglobin. Deferoxamine does not cause any demonstrable increase in the excretion of electrolytes or trace metals. In this use as an imaging drug, the chelate site is occupied by ^{89}Zr and is not displaced by serum iron.

5.2.1.1.1. At the Injection Site

Localized irritation; pain; burning; swelling; induration; infiltration; pruritus; erythema; wheal formation; eschar; crust; vesicles; local edema. Injection site reactions may be associated with systemic allergic reactions.

5.2.1.1.2. Hypersensitivity Reactions and Systemic Allergic Reactions

Generalized rash; urticaria; anaphylactic reaction with or without shock; angioedema.

5.2.1.1.3. Body as a Whole

Local injection site reactions may be accompanied by systemic reactions like arthralgia; fever; headache; myalgia; nausea; vomiting; abdominal pain; or asthma. Infections with *Yersinia* and *Mucormycosis* have been reported in association with Desferal use.

5.2.1.1.4. Cardiovascular

Tachycardia; hypotension; shock.

5.2.1.1.5. Digestive

Abdominal discomfort; diarrhea; nausea; vomiting.

5.2.1.1.6. Hematologic

Blood dyscrasia (thrombocytopenia; leucopenia).

5.2.1.1.7. Hepatic

Increased transaminases; hepatic dysfunction.

5.2.1.1.8. Musculoskeletal

Muscle spasms. Growth retardation and bone changes (e.g., metaphyseal dysplasia) are common in chelated subjects given doses above 60 mg/kg, especially those who begin iron chelation in the first three years of life. If doses are kept to 40 mg/kg or below, the risk may be reduced.

5.2.1.1.9. Nervous System

Neurological disturbances including dizziness; peripheral sensory, motor, or mixed neuropathy; paresthesias; seizures; exacerbation or precipitation of aluminum-related dialysis encephalopathy.

5.2.1.1.10. Special Senses

High-frequency sensorineural hearing loss and/or tinnitus are uncommon if dosage guidelines are not exceeded and if dose is reduced when ferritin levels decline. Visual disturbances are rare if dosage guidelines are not exceeded. These may include decreased acuity; blurred vision; loss of vision; dyschromatopsia; night blindness; visual field defects; scotoma; retinopathy (pigmentary degeneration); optic neuritis; and cataracts.

5.2.1.1.11. Respiratory

Acute respiratory distress syndrome (with dyspnea, cyanosis, and/or interstitial infiltrates).

5.2.1.1.12. Skin

Very rare generalized rash.

5.2.1.1.13. Urogenital

Dysuria; acute renal failure; increased serum creatinine and renal tubular disorders.

5.2.1.1.14. **Postmarketing Reports**

There are postmarketing reports of deferoxamine-associated renal dysfunction, including renal failure. Monitor subjects for changes in renal function (e.g., increased serum creatinine).

Combined, the previous experience in humans with antibodies labeled with ^{89}Zr supports the conclusion that a trial of labeled panitumumab at a mass dose of 1 mg, less than 7 nmole, approximately 1/420 of the usual clinical dose, will not pose a significant risk to the subjects in the proposed early clinical development.

9.2.6. 5.2.2 Expected Toxicities of Panitumumab-IRDye800

With the exception of infusion reactions and allergic responses, toxicities associated with panitumumab-based therapy are usually dose-dependent and associated with prolonged therapy. Because panitumumab will be delivered at sub-therapeutic doses and will only be administered once, it is anticipated that the primary adverse events will be dose-independent reactions such as infusion reaction or allergic reaction.

5.2.2.1 Dermatologic and Soft Tissue Toxicity (Black Box Warning)

Studies reporting dermatologic toxicities have given therapeutic doses of panitumumab repeatedly during treatment cycles since this is required to achieve a tumor response. In the proposed study we give one, sub-therapeutic dose.

In one study, dermatologic toxicities occurred in 90% of subjects and were severe (NCI CTC Grade 3 and higher) in 16% of subjects (package insert). The clinical manifestations included, but were not limited to, dermatitis acneiform; pruritus; erythema; rash; skin exfoliation; paronychia; dry skin; and skin fissures. Subjects who develop dermatologic or soft tissue toxicities should be monitored for the development of inflammatory or infectious sequelae. Life threatening and fatal infectious complications including necrotizing fasciitis; abscesses; and sepsis have been observed in subjects treated with panitumumab. Panitumumab should be withheld or discontinued for toxicities associated with severe or life-threatening inflammatory or infectious complications.

5.2.2.2 Infusion reaction (Black Box Warning)

Because the study proposes to administer a single sub-therapeutic dose, the most likely adverse event we anticipate is an infusion reaction. Minor (Grade 1) infusion reactions from panitumumab occur at a frequency of approximately 1%, with severe reactions (anaphylaxis) rarely reported (package insert). Serious infusion reactions can occur; requiring medical intervention and immediate, permanent discontinuation of panitumumab include rapid onset of airway obstruction (bronchospasm; stridor; and hoarseness); hypotension; shock; loss of consciousness; myocardial infarction; and/or cardiac arrest.

5.2.2.3 Cardiac Toxicity

Prolonged QT interval. Toxicology and other preclinical studies performed at UAB in 2013 with cetuximag-IRDye800 identified a small, but statistically significant increase in QT-intervals. These studies in non-human primates after administration of cetuximab-IRDye800 identified an increase in the QTc interval for cetuximab alone compared to cetuximab-IRDye800. There was also a statistically-significant difference between the QTc interval between the cetuximab and cetuximab-IRDye800 that will require assessment of interval ECG and exclusion criteria of subjects at risk for arrhythmias (see Exclusion Criteria). Screening ECG and intermittent ECG data will be obtained as part of the current study. Thus far, in our ongoing panitumumab-IRDye800 trials in HNSCC, such effects have not been seen (Stanford protocol IRB-35064).

5.2.2.4 Pulmonary Fibrosis / Interstitial Lung Disease (ILD)

Pulmonary fibrosis occurred in less than 1% of subjects in clinical studies using panitumumab. Subjects with a history or evidence of interstitial pneumonitis, pulmonary fibrosis, were excluded from most clinical trials (package insert). The estimated risk in a general population is therefore unknown. Cases of ILD, including fatalities, have been reported in subjects treated with panitumumab. Interrupt therapy for the acute onset or worsening of pulmonary symptoms.

5.2.2.5 Hypomagnesemia and Electrolyte Abnormalities

In one study, hypomagnesemia (NCI-CTC Grade 3 or 4) requiring oral or intravenous electrolyte repletion occurred in 2% of subjects. Hypomagnesemia occurred 6 weeks or longer after the initiation of panitumumab (package insert). Both hypomagnesemia and hypocalcemia occurred in some subjects. Electrolytes should be monitored periodically during treatment and for 8 weeks after the completion of therapy. Replete electrolytes as necessary using standard infusion guidelines.

5.2.2.6 Photosensitivity

Exposure to sunlight can exacerbate dermatologic toxicity (package insert). Advise subjects to wear sunscreen and hats and limit sun exposure while receiving panitumumab.

5.2.2.7 Ocular Toxicities

Keratitis and ulcerative keratitis, known risk factors for corneal perforation, have been reported with the use of panitumumab (package insert). Monitor for evidence of keratitis or ulcerative keratitis. Interrupt or discontinue therapy for acute or worsening keratitis.

5.2.2.8 Other Toxicities

It is also possible that other panitumumab related toxicities will appear (fatigue or diarrhea), but because the proposed dose is a fraction of the therapeutic dose and is administered only once, these are likely not to be the dose limiting toxicities in this clinical trial. We do not expect significant toxicities from IRDye800 because rodent toxicology studies have not demonstrated toxicity, and it has similar chemical structure as indocyanine green (which has been delivered to humans in gram quantities for decades without significant toxicity).

In one study, dermatologic toxicities occurred in 90% of subjects and were severe (NCI CTC Grade 3 and higher) in 16% of subjects (package insert). The clinical manifestations included, but were not limited to, dermatitis acneiform; pruritus; erythema; rash; skin exfoliation; paronychia; dry skin; and skin fissures. Subjects who develop dermatologic or soft tissue toxicities should be monitored for the development of

inflammatory or infectious sequelae. Life threatening and fatal infectious complications including necrotizing fasciitis; abscesses; and sepsis have been observed in subjects treated with panitumumab. Panitumumab should be withheld or discontinued for toxicities associated with severe or life-threatening inflammatory or infectious complications.

5.3 Availability

Panitumumab-IRDye800 will be provided by Stanford's investigational pharmacy. It has been manufactured at the GMP biologics facility at LI-COR Biosciences Inc. using cGMP IRDye800CW and commercially-available panitumumab. Formal testing of the panitumumab-IRDye800 for stability, sterility and antigen specificity is contained within the CMC section according to an FDA-approved process. The manufacturing and quality processes are documented elsewhere.

^{89}Zr-panitumumab will be manufactured at the MIPS Cyclotron and Radiochemistry Facility, at Stanford.

5.4 Agent Ordering

^{89}Zr-panitumumab

^{89}Zr-panitumumab will be manufactured on demand at the MIPS Cyclotron and Radiochemistry Facility. As necessary, ^{89}Zr-panitumumab will be held in appropriate storage until delivery to the clinical site.

Panitumumab-IRDye800

Per Stanford standard operating procedures, the Stanford investigational pharmacy will be responsible for storage of panitumumab-IRDye800 and dispensing of the drug after subjects are screened and enrolled to the study.

5.5 Agent Accountability

The Principal Investigator will be responsible for the accounting of ^{89}Zr-panitumumab and panitumumab-IRDye800, including proper labeling; documentation of lot numbers; dose and administration records; and storage of drug. The investigator will also be responsible for ensuring study drugs are correctly labeled and the appropriate dose is administered per the study protocol.

6. DOSE MODIFICATIONS

Apart from the dosing discussed in Section 4.3, there are no other dose modifications allowed in this study. If there is a limited range of tumor-to-background ratio identified in this study, we will consider amending the protocol to add higher doses of panitumumab-IRDye800 or ^{89}Zr-panitumumab.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Adverse Event

An adverse event is any event that is presents during the trial that was not observed at baseline or has worsened from baseline.

7.2 Serious Adverse Event

A serious adverse event is defined (21CFR§312.32) as any adverse experience that suggests a significant hazard, contraindication, side effect, or untoward medical occurrence that:

- Results in death,

- Is life-threatening (Note: the term “life-threatening” refers to an event in which the subject was at risk of death at the time of the event rather than to an event which hypothetically might have caused death if it were more severe),
- Requires (or prolongs) hospitalization,
- Causes persistent or significant disability/incapacity,
- Results in congenital anomalies or birth defects, or
- Other conditions which in the judgment of the Investigator represent significant hazards

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject, or may require intervention to prevent one of the other outcomes listed in the above definition. These should be considered serious.

Any pregnancy which occurs during this clinical trial must be reported to the Sponsor and treated as a serious adverse event with regard to the reporting time line. Any pregnancy must be followed through conclusion of the pregnancy (delivery or termination). Administration of study drug will be discontinued in a subject who becomes pregnant. If a male subject reports a pregnancy of his spouse or significant other while participating in the trial, data regarding the outcome of this pregnancy must be collected.

7.3 Assessment of Causality

For any adverse event that occurs during this study, it will be the responsibility of the Investigator to assess the relationship of the event to study treatment.
For this clinical trial, the following criteria will be used:

- *Unrelated:* There is no temporal relationship between the event and the administration of the study drug, and/or the event is clearly due to the subject’s medical condition, other therapies, or accident.
- *Possibly-related:* There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the participant’s medical condition or other therapies.
- *Probably-related:* The temporal relationship between the event and the administration of the study drug is compelling, and the participant’s medical condition or other therapies cannot explain the event.
- *Definitely-related:* The temporal relationship between the event and the administration of the study drug is compelling, the participant’s medical condition or other therapies cannot explain the event and the event follows a known or suspected response pattern to the medication.

Note that for this clinical trial, adverse reactions defined by the panitumumab package insert are considered “Expected” as cited by 21CFR§312.32(a).

7.4 Severity of Adverse Events

Adverse events will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0).

- Mild (Grade 1)
- Moderate (Grade 2)
- Severe (Grade 3)
- Immediately life-threatening/Fatal (Grade 4 and 5)

Adverse events that are solely laboratory test results without clinical consequence or sequelae or hospitalization will be graded by investigator judgement.

7.5 Safety Monitoring and Reporting

7.5.1 Monitoring of Adverse Events

Both serious and non-serious adverse events (AEs) will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each AE to determine whether it is unexpected according to the Informed Consent, Protocol Document, package insert, or cross-referenced Investigator's Brochure, and related to the investigation.

All adverse events (AEs), regardless of seriousness or relationship to study drug, are to be collected only on designated study dates from signing of informed consent through 30 days after administration of study drug, and recorded in the study specific worksheets, with the following exceptions:

- AEs that are solely laboratory values; are not related to the study drug; AND are clinically non-significant may not be collected.
- Non serious AEs that are not related to the study drug; are not clinically significant; AND are expected in the post-surgery clinical setting may not be collected.
- SAEs that occur from signing of informed consent to prior to administration of study agent may not be collected unless assessed as possibly, probably or definitely-related to a study procedure.

All patients enrolled in this study receive surgical intervention in the course of their standard of care. As a consequence of this surgery, patients may experience certain expected and normal adverse events. The following post-operative adverse events will be captured only in the patient's Electronic Health Record (EHR) source documentation and not in the adverse event log. If the adverse event exceeds the grading listed below or occurs outside the post-operative time window, the adverse event will be recorded in the Adverse Event Logs.

- Surgical wound site and associated, expected secondary signs and symptoms, including:
    - CTCAE v5.0 Grade 1 erythema, edema, and pruritis.
    - CTCAE v5.0 Grade 1 pain associated with surgical wound, including headaches – mild pain, little to no limit on adult daily life.
    - CTCAE v5.0 Grade 1 nausea, vomiting, and dysphagia.
    - CTCAE v5.0 Grade 1 weight loss, anemia, and electrolyte abnormalities.
    - CTCAE v5.0 Grade 2 trismus.

Whenever possible, symptoms should be grouped as single syndrome or diagnosis. The Investigator should specify the date of onset, maximal intensity, corrective therapy given, outcome, and his/her assessment of causality.

Any AE that is not resolved by the end of the study and considered to be potentially related to study drug or was the cause for the subject's withdrawal will be followed as clinically indicated until its resolution, or if non resolving, until considered stable. All SAEs will be tracked until resolution, or until 30 after the last dose of the study treatment.

7.5.2 Recording and Reporting Adverse Events and Serious Adverse Events

Non-serious adverse events will be reported annually to the FDA via an Annual Report and to the IRB via Continuing Review.

The PD (aka Principle Investigator) must immediately report within 24 hours of knowledge of event to the Sponsor-Investigator (i.e. IND Holder, Andrei Iagaru, MD), any serious adverse event, whether or not considered related to study drug. SAEs of all grades will be reported to the Sponsor-Investigator using the Stanford Cancer Institute SAE CRF. The investigator must provide a detailed description of the event, the treatment regimen, relevant laboratory and evaluations and assessment of the relationship of the AE to the study drug. All relevant information should be reported as it becomes available.

SAEs CTCAE Grade 3 and above, and all subsequent follow up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life threatening experiences.

Adverse events deemed serious, unexpected (i.e. not described in the protocol, package insert, cross-referenced Investigator's Brochure or informed consent documents) and related to study drug must be reported to Sponsor-Investigator using the FDA MedWatch form 3500a within 24 hours of knowledge of event. The Sponsor Investigator is responsible for deciding whether the event meets IND Safety Reporting criteria.

Events will be submitted to Sponsor Investigator Andrei Iagaru, MD, at:

-----Andrei Iagaru, MD, Professor of Radiology
Dept of Radiology, Division of Nuclear Medicine
Stanford Medicine at Stanford University M/C 5281
300 Pasteur Drive, Room H2200
Stanford, CA 94305
[redacted]

It will be the responsibility of the Sponsor-Investigator to provide to the FDA all information concerning significant hazards, contraindications, side effects or precautions felt significant to the safety of the drug being studied. The PD is responsible for accurate and timely communication to the Sponsor-Investigator of any events reportable to FDA. Subjects will be instructed prior to participation on the importance of reporting any symptoms (new or worsening), and/or any physical changes throughout their participation in the study. These events will be recorded accordingly in the CRF. Abnormal laboratory and/or clinical assessments will also be captured in the appropriate CRF.

8. CORRELATIVE/SPECIAL STUDIES

Conform our phase I study (IRB-35064), we will continue collecting pharmacokinetics data, hence the blood draws. We will also continue collecting imaging data on the primary tumor. This data will be used for: 1) drug delivery studies to gain better insight in the panitumumab-Irdye800 distribution in the tumor; and 2) to further study if the drug can be used to define (intraoperative) margins. Furthermore preoperative standard of care imaging obtained during routine evaluation will be used to correlate with pathological findings.

9. STUDY CALENDAR

9.1 Study Procedures

Enrolled subjects will follow the schedule of evaluations provided below.

Table 5: Study Procedures

	SCREENING/ PRE-TREATMENT (WITHIN 30 DAYS OF START OF TREATMENT)	DAY 0	DAY 1-4	DAY OF SURGERY (DAY 2 TO 5)	DAY 15 (± 4 days)	DAY 30 (± 4 days)
INFORMED CONSENT	X					
MEDICAL HISTORY	X					
PHYSICAL EXAMINATION [1]	X	X			X	
VITAL SIGNS	X	X		X	X	
HEIGHT AND WEIGHT	X					
PERFORMANCE STATUS [1]	X				X	
ECG	X [2]	X [3]			X	
CHEMISTRIES[4]	X	X		X	X	
HEMATOLOGY[5]	X				X	
TSH	X					
PTT/PT/INR	X					
SERUM PREGNANCY [6]	X	X				
PK–FLUORESCENT-LABELED ANTIBODY [7]		X	X	X	X	
PK-IMMUNOGENICITY [8]		X[8]			X	
IV INFUSION OF PANITUMUMAB-IRDYE800 [9]		X				
BOLUS INJECTION OF ^{89}ZR-PANITUMUMAB [10]		X				
SURGICAL RESECTION [11]				X		
TUMOR IMAGING	X [13]	X [12]	X [12]	X [14]		
PATHOLOGICAL EVALUATIONS [14]				X		
ADVERSE EVENTS [15]		X		X	X	X
CONCURRENT MEDICATIONS [16]	X	X	X	X	X	X

1. A physical examination will be done at baseline; standard head and neck examination and system-oriented physical examination will be conducted on Day 0 and Day 15. Examinations do not have to be repeated on Day 0 if completed within 21 days prior to infusion. Performance status assessment will be done at baseline and Day 15. Examinations collected for surgical procedure are acceptable for screening purposes.
2. An ECG will be obtained at screening to evaluate baseline. A previous ECG that was obtained as part of standard of care may be used as the baseline ECG if obtained within 21 days prior to of infusion.
3. On day of infusion, ECG will be collected 30 (±10) min after the end of panitumumab-IRDye800 infusion. Also an ECG will be obtained at the end of the 1 hour (±10 mins) observation period after completion of injection of ^{89}Zr-panitumumab.

4. Chemistries to include a standard basic metabolic profile with magnesium and phosphorus. Chemistries and other blood work collected for surgical procedure are acceptable for screening purposes. Chemistries do not have to be repeated on Day 0 if completed within 21 days prior to infusion.
5. Hematology (i.e., CBC) values include WBC; RBC; Hgb; hematocrit and platelet counts. Blood work collected for surgical procedure are acceptable for screening purposes.
6. A serum pregnancy test will be obtained on all women of childbearing age at baseline; a urine pregnancy test will be performed on Day 0 if the result of a serum pregnancy test within 72 hours before administration of panitumumab-IRDye800 is not available.
7. Blood samples for serum level of panitumumab-IRDye800 antibody and unconjugated dye, and ^{89}Zr-panitumumab antibody will be drawn as follows: on Day 0 prior to panitumumab-IRDye800 infusion and at the end of the 1-hour (± 10 mins) observation period after completion of injection of ^{89}Zr-panitumumab; on Day 1-4 (only one sample will be collected on the same day when the PETC/CT will be performed); on Day of surgery and on Day 15. The Day 15 time point will be 15 days ± 4 days, but is abbreviated as Day 15 throughout the protocol.
8. Blood will be collected on Day 0 prior to panitumumab-IRDye800 dose and on Day 15 and banked for future immunogenicity testing. Immunogenicity testing time points will not vary with dose.
9. Panitumumab-IRDye800 infusion to be given over 15 (±5 mins) minutes followed by 30 minutes (±10 mins) observation period.
10. ^{89}Zr-panitumumab administered as a bolus injection to be done after the end of the observation period after panitumumab-IRDye800 infusion; will be followed by 1 hour (± 10 mins) of observation period.
11. Surgical excision will be performed 2-5 days after the infusion of the study drugs.
12. A ^{89}Zr-PET/CT scan will be acquired after injection of ^{89}Zr-panitumumab. One scan will performed at any time starting on Day 0 (after end of observation period post ^{89}Zr-panitumumab administration) and until the day before surgery (inclusive). Subjects enrolled in the dosimetry sub-study will undergo three scans, performed at any time starting on Day 0 (after end of observation period post ^{89}Zr-panitumumab administration) and until the day before surgery (inclusive); if available, the scans will be performed on Day 0, Day 1 and on the day before surgery.
13. A standard of care ^{18}F-FDG-PET scan acquired within year from the day of study drug infusion is acceptable, even if this scan was/is acquired outside of Stanford.
14. Radiotracing and fluorescence imaging: Lymph node (and primary tumor) imaging will be done using the optical imaging devices (see Appendix B), in the clinic on Day 0 and at any other clinic visits between infusion and surgery if tumor is amenable to imaging and depending on subject availability. Radiotracer detection using the devices listed in Appendix C will be performed during surgery. Lymph nodes (and primary tumors) will be imaged intraoperatively to determine threshold of detection. Following surgery, pathological assessment of the specimens will take place.
15. See Section 7.5.1 for adverse events monitoring and reporting.
16. Concurrent medications will be recorded at screening. Medications administered on the Day 0 until Day 30 will not be collected unless given for a reportable adverse event or reportable serious adverse event.

9.2 Study Procedures

Subjects screened and enrolled will follow schedule of evaluations provided at the beginning of this protocol. The following evaluations will be performed:

9.2.1 Screening/Pre-Treatment (Within 30 days of treatment start)

- Obtain written informed consent
- Medical history including current medications
- Assessment of performance status
- Physical examination
- Vital signs to include blood pressure, heart rate, respiratory rate, temperature
- Height and weight

- Basic metabolic panel, serum magnesium and phosphorus
- CBC (WBC, RBC, Hgb, hematocrit and platelet counts)
- TSH
- PT/PTT/INR
- Serum pregnancy test for women of childbearing potential
- ECG
- Assessment of concomitant medications.
- A standard of care ^{18}F-FDG-PET scan acquired within a year from the day of study drug infusion is acceptable, even if this scan was/is acquired outside of Stanford.

NOTE: Labs and studies obtained for perioperative assessment can be used for screening evaluations.

9.2.2 Day 0

- **Prior to Infusion**
  - Vital signs (blood pressure, heart rate, respiratory rate, temperature)
  - Assessment of performance status (within 21 days)
  - Physical examination (within 21 days)
  - Chemistries (electrolytes, creatinine, BUN, magnesium, phosphorus) (within 21 days)
  - Pregnancy test for women of childbearing potential. If the result of a serum pregnancy test within 72 hours before administration of panitumumab-IRDye800 is not available, a urine pregnancy test will be performed on Day 0
  - Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody
  - Blood sample for future assessment of immunogenicity
- **IV infusion of panitumumab-IRDye800 over 15 minutes** (± 5 mins)
- **Vital signs immediately following panitumumab-IRDye800 infusion** (+ 5 mins)
- **Observation for 30 min** (± 10 mins)
- **ECG 30 min** (± 10 mins) **after the end of panitumumab-IRDye800 infusion**
- **Vital signs just before ^{89}Zr-panitumumab administration**
- **IV injection of ^{89}Zr-panitumumab bolus** followed by:
  - Vital signs immediately following ^{89}Zr-panitumumab administration (+ 5mins)
  - Observation for 1 hour (± 10 mins)
  - Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody collected at 1 hour (± 15 mins) after administration of ^{89}Zr-panitumumab
  - ECG will be obtained at 1 hour (± 15 mins) after administration of ^{89}Zr-panitumumab
  - Assessment for adverse events at the end of the 1 hour observation period
- DOSIMETRY STUDY ONLY:
  - ^{89}Zr-panitumumab whole body PET/CT imaging after completion of observation

9.2.3 Day 1 to Day before Surgery:

- The following assessments will performed once, at any time between Day 1 and the day before surgery (including on Day 1 and on the day before surgery):
  - Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody.
  - ^{89}Zr-panitumumab whole body PET/CT imaging.

- Assessment for adverse events.
- Assessment for concomitant medications.
- Imaging using the optical and radiotracer imaging/detection devices in the clinic to assess fluorescence and radiotracer intensity if tumor amenable to imaging and depending on subject availability.

- DOSIMETRY STUDY ONLY:
  - A total of two ^{89}Zr-panitumumab whole body PET/CT scans will performed at any time between Day 1 and the day before surgery (including on Day 1 and on the day before surgery if possible); if available, the scans will preferably be performed on Day 1 and on the day before surgery.
  - Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody will be collected on the same days when PET/CT imaging is performed.

9.2.4. Day 2 to 5 (Day of Surgery)

- Prior to surgery: Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Chemistries (electrolytes, creatinine, BUN, magnesium, phosphorus)
- Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody
- Scheduled routine surgical resection, per standard of care
- Intraoperative imaging using the optical and radiotracer imaging/detection devices (if accessible for imaging; Appendix B and C)
- Pathological evaluations – tissue assessments of the pathological tissues by optical scanning after clinical assessment by pathologist but prior to permanent fixation of the tissue
- Assessment for adverse events
- Assessment for concomitant medications

9.2.5. Day 15 (± 4 days)

- Physical examination
- Performance Status
- Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Chemistries (electrolytes, creatinine, BUN, magnesium, phosphorus)
- CBC
- Assessment for adverse events
- Assessment for concomitant medications
- Blood sample for panitumumab-IRDye800 antibody and ^{89}Zr-panitumumab antibody and immunogenicity
- ECG

9.2.6 Day 30 (± 4 days)

- Investigator or study staff will contact the subject by telephone or review of electronic medical record data to collect any additional adverse events that are attributable to the study and/or concomitant medications.

9.3 Description of Evaluations / Procedures

9.3.1 Laboratory Evaluations

Blood specimens will be collected for hematology (WBC, RBC; hemoglobin, hematocrit, and platelet counts), basic metabolic profile electrolytes, creatinine, BUN, magnesium and phosphorus, and serum pregnancy if necessary. If laboratory evaluations are obtained within 21 days of Day 0, they do not have

to be repeated. Blood will be collected for fluorescent-labeled antibody pharmacokinetics and furthermore, blood will be obtained and banked for future analysis of immunogenicity against the study drug as requested by the FDA in pre-IND conversations.

9.3.2 Physical Examinations

A physical examination will be conducted to include review of all major systems prior to enrollment to study. If the initial physical examination is obtained within 21 days of Day 0, the exam does not have to be repeated. Standard head and neck examination and system-oriented physical examination will be conducted on other visits.

9.3.3 Medical History

A complete medical history including age, sex, race, current medications, any current or previous medical conditions, any previous surgeries, smoking history, and alcohol use will be done at screening.

9.3.4 Vital Signs

Vital signs including blood pressure, heart rate, respiratory rate, and temperature will be collected at each clinic visit. Height and weight will be collected at screening.

9.3.5 Tumor and lymph node imaging

Imaging will be done utilizing the optical imaging devices (see Appendix B for details). The camera head will be placed over the tumor and/or lymph nodes and images will be obtained and recorded.

9.3.6 Cardiac Monitoring

Preclinical studies performed at UAB (2013) identified a small, but statistically significant increase in QT-intervals using cetuximab. These studies in non-human primates after administration of cetuximab-IRDye800 identified an increase in the QTc interval for cetuximab alone compared to cetuximab-IRDye800. There was also a statistically significant difference between the QTc interval between the cetuximab and cetuximab-IRDye800 that will require assessment of interval ECG and exclusion criteria of patients at risk for arrhythmias, see exclusion criteria. Screening ECG and intermittent ECG data will be obtained as part of the study. We reference the full toxicology report for Cetuximab-IRDye800 (IND 115706) recently submitted to the FDA. However, more recent investigation (40) has failed to substantiate this QTc risk in panitumumab-IRDye800 and has further shown no significant evidence of cardiac toxicity.

An ECG will be obtained during the screening period, on day of infusion (at 30 min (±10) after the end of panitumumab-IRDye800 infusion and at the end of the 1 hour observation period after completion of injection of ^{89}Zr-panitumumab) and on Day 15. A previous ECG that was obtained as part of standard of care may be used as the baseline ECG if obtained within 21 days prior to injection and will be reviewed any time prior to eligibility. QT/QTc interval will be assessed for each time point. A supervising physician will review the ECG results the same day they are recorded, the final review can be delayed (pending on availability of final reading) but initial review for safety is done the same day.

10. MEASUREMENTS

10.1 Primary Outcome Measure

The number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were predicted to be tumor-positive by ^{89}Zr-panitumumab labeling.

We will compare the number and location of ^{18}F-FDG-avid nodes within the regional lymphatics to the number and location of ^{89}Zr-panitumumab-avid nodes in relation to the postoperative neck dissection histopathology (gold standard).

Outcome Measure: Preoperatively, the location and number of ^{18}F-FDG-avid nodes as well as the number and location of ^{89}Zr-panitumumab-avid nodes will be determined by 2 board-certified Nuclear Medicine physicians (Dr. Guido Davidzon and Dr. Andrei Iagaru). Once all radioactive and fluorescent nodes are removed intraoperatively, standard of care will be

performed via completion neck dissection. The excised tissue specimens will be sent to pathology where the tumor status of the lymph nodes will be determined (histology serves as our gold standard). This will allow us to determine true and false positive and negative tumor values for ^{89}Zr-panitumumab and ^{18}F-FDG in identifying the tumor-loaded lymph nodes.

Time Frame: Final false positive and negative rate, sensitivity and specificity of sentinel node detection using ^{89}Zr-panitumumab can be determined towards the completion of the trials (once all subjects have been included and all the data has been collected).

Safety Issue: No

10.2 Secondary Outcome Measures

The number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were <u>NOT</u> predicted tumor-positive by ^{89}Zr-panitumumab labeling.

We will compare the number and location of ^{18}F-FDG-avid nodes within the regional lymphatics to the number and location of ^{89}Zr-panitumumab-avid nodes in relation to the postoperative neck dissection histopathology (gold standard).

Outcome Measure: Preoperatively, the location and number of ^{18}F-FDG-avid nodes as well as the number and location of ^{89}Zr-panitumumab-avid nodes will be determined. Once all radioactive and fluorescent nodes are removed intraoperatively, standard of care will be performed via completion neck dissection. The excised tissue specimens will be sent to pathology where the tumor status of the lymph nodes will be determined (histology serves as our gold standard). This will allow us to determine true and false positive and negative tumor values for ^{89}Zr-panitumumab and ^{18}F-FDG in identifying the tumor-loaded lymph nodes. Specifically we will look for lymph nodes that were ^{18}F-FDG positive on preoperative imaging and/or fluorescently labeled by panitumumab-IRDye800, but do now show uptake of ^{89}Zr-panitumumab.

Time Frame: Final false positive and negative rate, sensitivity and specificity of sentinel node detection using panitumumab-IRDye800 can be determined towards the completion of the trial (once all subjects have been included and all the data have been collected).

Safety Issue: No

11. REGULATORY REQUIREMENTS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators. The protocol and any significant amendments will be submitted to FDA.

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include annual review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly (monthly) review any DLTs, serious adverse events, and protocol deviations associated with the research to ensure the protection of human subjects. Results of

the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director, or designees noted in the Delegation of Authority Log, will maintain adequate and accurate participant case histories with observations and other data pertinent to the study. Original source documents will be transcribed to Case Report Forms (CRFs). Source documents include hospital records, clinical charts, laboratory and pharmacy records, and recorded electronic data. Case report forms will be developed using the OnCore database system and maintained by the study team. CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

Data from both cohorts (14 subjects) will be combined for all primary and secondary analyses.

Primary objective

Determine the sensitivity and specificity of ^{89}Zr-panitumumab for the detection of tumor-involved regional lymph nodes.

Using the histological and/or pathological evaluation of the lymph node as the gold standard, we will calculate and compare the sensitivity and specificity of ^{89}Zr-panitumumab and ^{18}F-FDG PET/CT imaging findings. We will test the level of association of the ^{89}Zr-panitumumab-PET results and the ^{18}F-FDG-PET results with the Phi coefficient.

We will use the matched pair Durkalski's test (36), accounting for the cluster-correlation within subjects, for the sensitivity and specificity comparisons (primary and exploratory) using the clust.bin.pair package (37) in the R-statistical software (39). Significance for both primary and exploratory analyses will be assessed at the 0.05 level and to assess uncertainty, 95% confidence intervals will be provided for all estimates.

Secondary Objective

Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were NOT predicted tumor-positive by ^{89}Zr-panitumumab labeling.

We will calculate the odds of a false negative using ^{89}Zr-panitumumab-PET relative to the odds of a false negative using a ^{18}F-FDG-PET and compare the negative predictive value (NPV) of ^{89}Zr-panitumumab and ^{18}F-FDG.

Exploratory Objectives

- **Determine the sensitivity and specificity of panitumumab-IRDye800 for the detection of tumor-involved regional lymph nodes.**
- After isolation of lymph nodes from the specimen in the pathology lab, lymph node fluorescence will be measured prior to histological and/or pathological evaluation (gold standard). Sensitivity and specificity will be generated by comparison of fluorescence intensity and histological and/or pathological evaluation (gold standard). Findings will furthermore be compared to the sensitivity and specificity of ^{89}Zr-panitumumab and ^{18}F-FDG (primary objective) using Durkalski's test, as outlined above.

- **Determine the number (proportion) of lymph nodes determined to be tumor-positive by histological and/or pathological evaluation that were NOT predicted tumor-positive by panitumumab-Irdye800 labeling.**
- We will determine the level of association between panitumumab-Irdye800 results and ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET results with the Phi coefficient.

Descriptive statistics

For qualification and quantification of the fluorescence signal and the radioactive signal, we follow the following rules:

- *Intraoperative fluorescence assessment of panitumumab-IRDye800:*
  There will be no set level of fluorescence whereby we consider tissue positive or negative; it is based on operator's impression.
- *Ex vivo fluorescence assessment of panitumumab-IRDye800:*
  From the fluorescence images taken in the operation room, we will calculate tumor-to-background ratios (TBRs) as such to define a more quantitative read-out (similar to as described in the IRB-35064 study). Based on the current data available from our IRB-35064, in our 50 mg cohort, we found an average TBR of 6.53±1.2, a sensitivity of 89±5.1%, a specificity of 92±3.5% (AUC: 0.93±0.05). The NPV and PPV were 94±2.8% and 92±9.5%, respectively. We will extrapolate these findings to the current study to further validate the findings from our phase I study (IRB-35064).
- *Radioactive assessment of ^{89}Zr-panitumumab:*
  Following the standard of care evaluation of PET/CT scans, maximum standard uptake values (SUV_{max}) will be calculated for the accumulation of ^{89}Zr-panitumumab in tumor tissue and/or lymph nodes. As background reading, the SUV_{max} of the mediastinum will be determined.
  Tentative cutoff for 'imaging positive': an SUV_{max} value of the lymph nodes of minimally 1.4x the background (mediastinum). In all cases, (histo-) pathology serves as the gold standard for "true" positive / negative lymph nodes. Subsequently an ROC curve will be generated whereby we will select the point of highest specificity and sensitivity and from there we will determine if our tentative cutoff was valid or if we need to adjust.
- *Comparison fluorescence and radioactivity-based findings:*
  All tissues that will be excised during surgery will be evaluated for the presence of radioactivity and fluorescence and scored as such being positive / negative. Evaluated tissues are either 1) fluorescent only; 2) radioactive only; 3) both radioactive and fluorescent; or 4) neither radioactive nor fluorescent. This will be done quantitatively (Y/N approach) and qualitatively (see above).
    - Because the antibody is the same, only the label (IRDye800 or 89-Zr) differs, we expect that tissue samples that are radioactive are also fluorescent and vice versa. However, this is something that will be investigated in this study.

We will present means and standard deviations (or medians and interquartile ranges when appropriate) for continuous characteristics such as maximum standard uptake values (SUV_{max}) as determined with ^{89}Zr-panitumumab-PET and ^{18}F-FDG-PET and radioactivity counts of ^{89}Zr-panitumumab in the node(s) as determined with the beta probe and/or high-energy gamma probe, and tumor-to-background ratios (TBRs) and mean florescence intensity (MFI) for panitumumab-Irdye800. For categorical variables such as drainage pattern and number of lymph nodes visualized, we will present frequency statistics. Graphical tools such as heat maps, histograms, and boxplots will be used to assess distributional properties of continuous variables. We will illustrate the nature of the relationship between SUV_{max}/radioactivity counts and/or MFI/TBRs and/or drainage pattern across all subjects, the relationship will be illustrated with a heat map and spaghetti plot. When appropriate, transformations – including a log-based transformation – may be considered to stabilize variance

Sample Size Justification and Power Considerations

Since this is an early Phase I study, power considerations are limited but even so, we have excellent power to detect differences in specificity. With 14 subjects and an average of 30 nodes per subject, we expect 420 specimens on which to calculate and compare sensitivity and specificity. However, in calculating power, we must account for the clustering or correlation of lymph nodes within subject. Although we will use Durkalski's test to compare sensitivities and specificities, we estimated power by first determining the effective sample size (ESS), adjusting for the correlation of LNs within person, and then estimated power using McNemar's test on this ESS. We assumed an intra-class correlation of 0.005, yielding an effective sample size of 367 LNs. With an effective sample size of 367, a prevalence of 7.5%, a significance level of 0.05, and a 0.3 proportion of discordant pairs, we have greater than 95% power to detect a 0.1 difference in specificities, assuming the lower specificity is 0.7. Similarly, we have greater than 71% power to detect a 0.25 difference in sensitivity with the one-sided McNemar test. This assumes the proportion of discordant pairs is 0.3 and the lower sensitivity is 0.7.

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APPENDICES

APPENDIX A: **Participant Eligibility Checklist**

A Participant Eligibility Checklist will be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist will be retained in the subject's study file and the study's Regulatory Binder.

The study coordinator, treating physician and an independent reviewer will verify that the participant's eligibility is accurate, complete, and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

Protocol Title:	**Pilot study evaluating Panitumumab-Irdye800 and ^{89}Zr-Panitumumab for dual-modality imaging for nodal staging in head and neck cancer**
Protocol Number:	**IRB-41878 / OnCore# ENT0066**
Principal Investigator:	**Andrei Iagaru, MD**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

III. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. Biopsy-confirmed diagnosis of squamous cell carcinoma of the head and neck	☐	☐	
2. Subjects diagnosed with any T stage, any subsite within the head and neck that are scheduled to undergo surgical resection. Subjects with recurrent disease or a new primary will be allowed	☐	☐	
3. Planned standard of care surgery with curative intent for squamous cell carcinoma	☐	☐	
4. Age ≥ 19 years	☐	☐	
5. Have acceptable hematological status, coagulation status, kidney function, and liver function including the following clinical results: -Hemoglobin ≥ 9gm/dL -White blood cell count > 3000/mm^3 -Platelet count ≥ 100,000/mm^3 -Serum creatinine ≤ 1.5 times upper reference range	☐	☐	

Exclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
1. Myocardial infarction (MI); cerebrovascular accident (CVA); uncontrolled congestive heart failure (CHF); significant liver disease; or unstable angina within 6 months prior to enrollment	☐	☐	
2. Previous bilateral neck dissection.	☐	☐	
3. History of infusion reactions to other monoclonal antibody therapies	☐	☐	
4. Pregnant or breastfeeding	☐	☐	
5. Magnesium or potassium lower than the normal institutional values	☐	☐	
6. Subjects receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents	☐	☐	
7. Subjects with a history or evidence of interstitial pneumonitis or pulmonary fibrosis	☐	☐	
8. Severe renal disease or anuria			
9. Known hypersensitivity to deferoxamine or any of its components	☐	☐	

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

APPENDIX B: Optical Imaging Modalities: Overview

All of the non-significant risk (NSR) devices listed below, in the determination of the Investigator, meet the following criteria when used as proposed in this study:

- Are not implanted
- Do not support or sustain human life
- Are not of substantial importance for diagnosis, curing, mitigating or treating disease; or otherwise preventing impairment of human health
- Do not present any other potential for serious risk to the health, safety or welfare of subjects

Device name	Manufacturer	Commercially available?	510(k)	NSR?	Serial No. of submission to [redacted]
Intraoperative near-infrared fluorescence imaging, in vivo					
Spy-Phi– and/or PINPOINT – R9000 (Handheld/Endoscopic)	Novadaq Inc.	Yes (modified light source)	No	Yes	SN0000
Explorer Air	SurgVision	Yes (modified light source)	No	Yes	SN0000
PDE-NEO II and/or FIGS	Hamamatsu Photonics KK.	Yes	Yes	N/A	SN0000
IMAGE1 S RUBINA System*	Karl Storz	Yes	N/A	Yes	SN0017
Intraoperative near-infrared fluorescence imaging, ex vivo (back table)					
Spy-Phi– and/or PINPOINT – R9000 (Handheld/Endoscopic)	Novadaq Inc.	Yes (modified light source)	No	Yes	SN0000
Explorer Air	SurgVision	Yes (modified light source)	No	Yes	SN0000
IGP ELVIS v4	LI-COR Biosciences	No	No	Yes	SN0000
PDE-NEO II and/or FIGS	Hamamatsu Photonics KK.	Yes	Yes	N/A	SN0000
IMAGE1 S RUBINA System*	Karl Storz	Yes	N/A	Yes	SN0017

Device name	Manufacturer	Commercially available?	510(k)	NSR?	Serial No. of submission to [redacted]
Laboratory near-infrared fluorescence imaging, ex vivo					
Pearl Triology	LI-COR Biosciences	Yes			
Odyssey CLx	LI-COR Biosciences	Yes			
IGP ELVIS v4	LI-COR Biosciences	No			
Leica fluorescence microscope	Leica	Yes			
Vevo LAZR-X	Visualsonics	Yes			
Firefly stand-alone device	Intuitive Surgical Inc.	Yes			

* Please refer to Appendix D for IMAGE1 S RUBINA System Karl Storz Components Overview

APPENDIX C: Imaging Modalities for the Detection of ^{89}Zr: Overview

All of the non-significant risk (NSR) devices listed below, in the determination of the Investigator, meet the following criteria when used as proposed in this study:

- Are not implanted
- Do not support or sustain human life
- Are not of substantial importance for diagnosis, curing, mitigating or treating disease; or otherwise preventing impairment of human health
- Do not present any other potential for serious risk to the health, safety or welfare of subjects

Device name	Manufacturer	Commercially available?	510(k)	NSR?	Serial No. of submission to [redacted]
Intraoperative imaging, in vivo					
Beta probe + Node Seeker	IntraMedical Imaging	Yes	Exempt, class I device	No	N/A
Neoprobe	Leica Biosystems	Yes	Yes, K971167	No	N/A
Crystal probe	Crystal Photonics	Yes	Exempt, class I device	No	N/A
Ex vivo imaging (back table)					
Beta probe + Node Seeker	IntraMedical Imaging	Yes	Exempt, class I device	No	N/A
Neoprobe	Leica Biosystems	Yes	Yes, K971167	No	N/A
Crystal probe	Crystal Photonics	Yes	Exempt, class I device	No	N/A
Beta camera	IntraMedical Imaging	No	Not yet applied for	N/A	N/A
Laboratory imaging					
Beta camera	IntraMedical Imaging	No			
Cherenkov imager (LightPath)	LightPoint Medical	Yes			
1 mm resolution mobile PET scanner	Dr Craig Levin (in-house design)	No			

APPENDIX D: IMAGE1 S RUBINA System Karl Storz Components Overview

Material	Description	Quantity	CE-certified	FDA-approved
TM340	32" 4K Monitor	1	X	X
TC201	IMAGE1 S CONNECT II, connect module	1	X	X
TC304	IMAGE1 S 4U-LINK, link module	1	X	X
TL400	Cold Light Fountain Power LED Rubina	1	X	X
TH121	IMAGE1 S 4U RUBINA, OPAL1 NIR/ICG, two-chip 4K UHD camera head	1	X	X
495NCSC	Fiber Optic Light Cable	2	X	X
26003ACA	HOPKINS Straight Forward Telescope 0°, enlarged view, diameter 10 mm, length 31 cm	1	X	X
8711AGA	HOPKINS Straight Forward Telescope 0°, NIR/ICG, ø 10mm, 20cm	1	X	X
8710AGA	HOPKINS Straight Forward Telescope 0°, NIR/ICG, diameter 5.8 mm, length 20 cm	1	X	X
28272 CN/UGK/HC	Components for mechanical holding arm	1	X	X
28172 HM/HR	Components for mechanical holding arm	1	X	X
28164AC	HOPKINS Forward Telescope 0°, enlarged view, diameter 4 mm, length 18 cm	1	X	X
28614BC	HOPKINS Forward Telescope 30°, enlarged view, diameter 4 mm, length 18 cm	1	X	X
28614FC	HOPKINS Forward Telescope 45°, enlarged view, diameter 4 mm, length 18 cm	1	X	X
20916025 AGA	VITOM II NIR/ICG Telescope 0°	1	X	X
20100032	Fluorescein Blue Filter System, for fluorescence diagnosis	1	X	X
20100033	Fluorescein Barrier Filter	1	X	X