A Phase 2 Study Evaluating Panitumumab-IRDye800 vs Sentinel Node Biopsy and (Selective) Neck Dissection for Metastatic Lymph Node Identification in Patients with Head and Neck Cancer

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Document History	
Version Date	**Notes**
29 September 2017	Version submitted to IRB
26 October 2017	SRC Pre-Review Comments
30 November 2017	SRC Comments + Adding Device Appendix
3 October 2018	• Clarification of cohort structure and recruitment based on NCCN guidelines version January 2018 for use of Sentinel Node Biopsy (SNB) in head and neck cancer. (Cohort 1 – T1-2 and N0, Cohort 2 – any T and N+) • Clarification that panitumumab-IRDye800 infusion will be 1 to 5 days before surgery, not 2 to 5 days before surgery • Clarification of the rationale for use of panitumumab-IRDye800 • Addition of additional description/clarification of SNB and neck dissection procedure for cohort 1 and 2 respectively. • AEs will be graded according to CTCAE v5.0, not 4.03 • Clarification of additional PKs to be collected on days 1-3 post-operation per availability up to once per day on days 1, 2, and 3 post-operation. • Removed Nicholas Oberhelman as study coordinator • Updated fluorescence imaging device (Appendix B) list to reflect incorporation of upgraded devices: o Spy-Phi for intraoperative in vivo and ex vivo imaging o FIGS for intraoperative in vivo and ex vivo imaging o (Stand-alone) da Vinci Firefly for intraoperative ex vivo imaging and laboratory imaging o VEVO LAZR-X for intraoperative in vivo and ex vivo imaging and laboratory imaging o Removal of SPY/LUNA imaging devices
26 September 2019	• Change of Principal Investigator / Protocol Director from Eben Rosenthal t Fred Baik. Dr Rosenthal is now listed as co-investigator. • Personnel update: Included Roan Raymundo and Grace Yi as study coordinators Other personnel changes are only made in eProtocol. • Protocol modifications: - Removal of "Received an investigational drug within 30 days prior to first dose of panitumumab-IRDye800" as an exclusion criteria (Section 3.2 and Appendix A). - Addition of photoacoustic imaging as correlative study (as discussed in IRB-35064). - Update Appendix B to reflect the most recent fluorescence imaging devices (also updated in eProtocol). - Administrative changes.

15 November 2019	• Administrative changes - Changed Dr. Eben Rosenthal (now co-I) to Dr. Fred Baik in Appendix A: Participant Eligibility Checklist - Changed "Sponsor-Investigator" to "Sponsor" in Section 7 and Section 11.1
9 December 2019	• Removed Stefania Chirita as CRC, addition of Roan Raymundo as CRC
1 February 2021	• Based on published Panitumumab-IRDye800 QTc interval safety data, Day 0 and Day 15 ECGs have been removed • Clarified expected Adverse Event collection window to only designated study dates • Adverse Event and Serious Adverse Event reporting clarification • Day 1 to 3 Post-operative (optional visit) has been removed from Study Calendar

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PROTOCOL SYNOPSIS

TITLE	**A Phase 2 Study Evaluating Panitumumab-IRDye800 vs Sentinel Node Biopsy and (Selective) Neck Dissection for Metastatic Lymph Node Identification in Patients with Head and Neck Cancer**
STUDY PHASE	Phase 2
INDICATION	Clinically-suspected or biopsy-proven diagnosis of head and neck squamous cell carcinoma (HHSCC). Subjects diagnosed at any T stage who are scheduled to undergo surgical resection with a neck dissection or sentinel node biopsy (SNB) as part of standard of care are eligible.
INVESTIGATIONAL PRODUCT	A panitumumab-IRDye800 dose of 50 mg, will be systemically infused 1 to 5 days before surgery. Panitumumab-IRDye800 has been manufactured under current good manufacturing practice (cGMP) by LICOR Biosciences Inc. Eben Rosenthal (co-PI) currently holds the IND (119474). On the day before surgery 2 mCi Lymphoseek (^{99m}Tc-tilmanocept) will be administered locally around the primary tumor for sentinel node mapping. Lymphoseek is obtained in unit doses from Cardinal Health.
TREATMENT SCHEDULE	A panitumumab-IRDye800 dose of 50 mg, will be systemically infused 1 to 5 days before surgery. On the day before surgery, to subjects undergoing sentinel node biopsy, the standard dose of ~2 mCi Lymphoseek will be administered locally around the primary tumor after which lymphoscintigraphy and SPECT/CT imaging will be performed per standard of care to determine the number and location of foci of radiopharmaceutical uptake indicating sentinel nodes. On the day of surgery, intraoperative fluorescence imaging will be performed using an intraoperative optical imaging device (a list of devices is provided in Appendix B). Per standard of care, for the subjects undergoing sentinel node biopsy, a gamma probe will be used to search for radioactive lymph nodes as well. *Ex vivo* fluorescence imaging and gamma counting of the excised specimens will be performed prior to and after pathological assessment. At all steps, equipment will be used that does not violate or destroy the pathological tissues.
PRIMARY OBJECTIVE(S)	The primary objective of the study is to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck lymph nodes of subjects with head and neck squamous cell carcinoma (HNSCC).
SECONDARY OBJECTIVE(S)	Determine if panitumumab-IRDye800 can identify sentinel nodes with the same accuracy as Lymphoseek.

SAMPLE SIZE AND STUDY DURATION	It is anticipated that 20 subjects will be enrolled into the study. Each subject will be followed for 30 days following the last dose of study medication. Based on the enrollment rate from the panitumumb-IRDye800 trial in HNSCC, it is anticipated that the study will require 2 years to be completed.
STATISTICAL CONSIDERATIONS	To answer the **primary objective** we will calculate the sensitivity and specificity of the intraoperative findings on whether the lymph node is fluorescent (ie, presence of panitumumab-IRDye800) to the histopathological (gold standard) status of the lymph nodes. Analysis for the **secondary objective:** We will determine if panitumumab-IRDye800 can identify sentinel nodes with the same accuracy as Lymphoseek. Similar to the primary analysis, we will calculate the specificity and sensitivity of Lymphoseek for metastatic lymph node identification, using histology as the gold standard, and compare these to the fluorescence imaging-based results. Since false negatives are of particular concern, we will calculate the diagnostic odds ratio to better understand the odds of obtaining a false negative using panitumumab-IRDye800 relative to the odds of obtaining a false negative using Lymphoseek-based sentinel node biopsy.

STUDY SCHEME

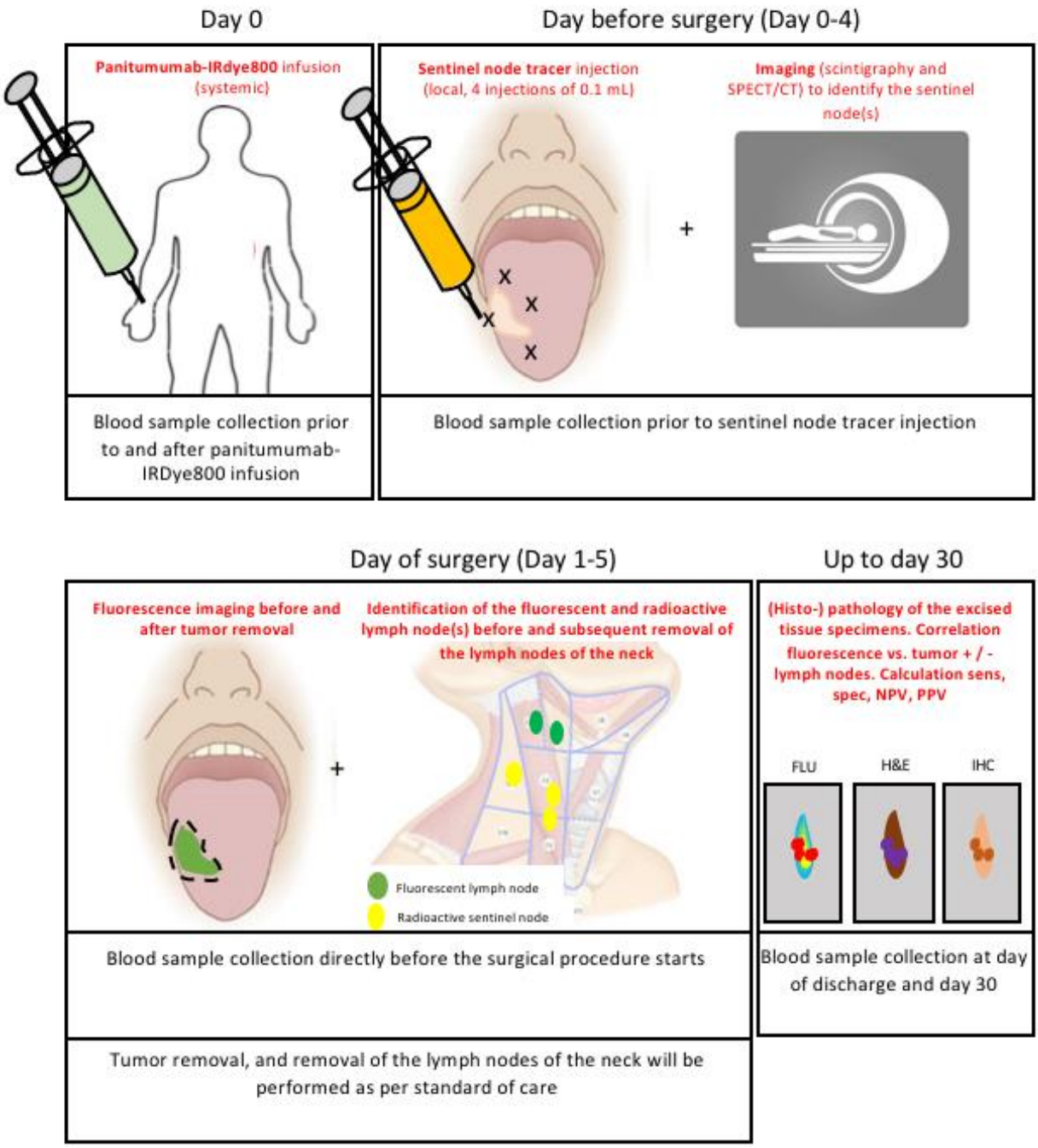


Figure 1. Schematic overview of the clinical trial in which we will directly compare panitumumab-IRDye800 to the reference standards sentinel node biopsy and neck dissection. Key steps of the clinical study are mentioned.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

^{99m}Tc	Technetium-99m
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
CT	Computed Tomography
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
eLND	Extensive Lymph Node Dissection
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
Hgb	Hemoglobin
HNSCC	Head and Neck Squamous Cell Carcinoma
HTN	Hypertension
ICG	Indocyanine green
ILD	Interstitial Lung Disease
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower Limit of Normal
LN	Lymph Node
MTD	Maximum Tolerated Dose
NIR	Near-Infrared
NHS	N-hydroxysuccinimide
PAI	Photoacoustic imaging
PLT	Platelet
ROI	Region of Interest
SAE	Serious Adverse Event
SN	Sentinel Node
SNB	Sentinel Node Biopsy
SNR	Signal to Noise Ratio
SPECT	Single Photon Emission Computed Tomography
TBR	Tumor to Background Ratio
ULN	Upper Limit of Normal
UNK	Unknown
WBC	White Blood Cell
WHO	World Health Organization

1. OBJECTIVES

1.1. Primary Objective

The primary objective of the study is to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck lymph nodes of subjects with head and neck squamous cell carcinoma (HNSCC).

1.2. Secondary Objective

Determine if panitumumab-IRDye800 can identify sentinel nodes with the same accuracy as Lymphoseek.

2. BACKGROUND

2.1 Study Disease

Squamous cell carcinoma of the head and neck affects more than 61,760 people in the United States annually, with a 5-year survival rate being <60% (1,2). **The presence of lymph node (LN) metastasis is considered the most important negative prognostic factor for survival in patients with primary HNSCC** (3,4). Despite advanced anatomic and metabolic imaging techniques, occult LN (micro-) metastasis remain in the clinically and radiographically negative neck (cN0). In fact, 10 to 30% of cN0 patients present with occult LN metastasis (4). Even more so, treatment of the neck is often indicated when the risk of LN metastasis exceeds 15 to 20% (5,6). To address this surgically, the current NCCN guidelines recommend either elective regional neck node dissection or sentinel node (SN) biopsy (SNB) (7).

Elective lymph node dissection causes significant morbidity: The gold standard for detection of regional metastatic disease in HNSCC traditionally has been a (elective) neck dissection. However, shoulder dysfunction and pain due to accessory nerve (cranial nerve XI) injury are common after neck dissection (8–11). 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 (12). To reduce morbidity, there has been a gradual migration away from extensive dissection to more limited dissection offered by SNB where only the primary tumor draining LN(s) are removed for detailed histologic examination (13–18). SNB has already replaced regional lymphadenectomy in melanoma and breast surgery to avoid the associated deficits associated with this more aggressive approach, and is now also advocated for HNSCC (7).

Sentinel node biopsy: SNB is performed as a surgical staging method to detect occult nodal disease in those tumor types with a high metastatic rate and where LN disease predicts outcome and informs adjuvant treatment decisions. Assuming the orderly spread of tumor cells through the lymphatic system (19), excision and evaluation of the primary tumor-draining LNs only (so-called SN(s) will allow accurate determination of the tumor status of the regional LNs in the neck. SNB thus allows the selection of node-positive subjects that will benefit from additional surgical or adjuvant therapy, while sparing node-negative patients an unnecessary neck dissection.

Typically, to identify the SN(s) preoperatively, a local injection of a non-tumor specific radiotracer is given around the primary tumor followed by lymphoscintigraphy to visualize

drainage of the radiotracer through the lymphatic system to the SN(s) (20–22). The addition of single photon emission computed tomography combined with computed tomography (SPECT/CT) was shown to allow for localization of the SN within its anatomical context, providing the surgeon with a 3D map to plan the surgical procedure (22). During the surgical procedure, these SN(s) can then be identified and excised using a gamma ray detection probe that localizes the radioactive signal in the nodes (20,23). More recently, mobile gamma cameras were introduced to provide the surgeon with additional (visual) feedback with regard to having localized and removed the radioactive LNs (24).

Meta-analysis of SNB in breast cancer, head and neck cancer, and melanoma have validated the accuracy of SNB in predicting occult LN metastasis (13,17,25,26). More recently, for HNSCC, the validity of the approach was also shown. In a large multicenter study including 227 SNB procedures from 6 centers, Alkureishi *et al*., showed that the procedure was successful in 93% subjects whereby in 34% (42 subjects) of cases HNSCC subjects were upstaged whereby in 10 subjects only micro-metastasis were present (27). Where the centers participating in the Alkureishi *et al*., paper used colloid-based SN tracers, more recently a mannose-receptor targeting tracer for SNB was introduced, Lymphoseek (28–34). In head and neck cancer, using Lymphoseek an even higher sensitivity was reported: in 98% of subjects (81/83) at least one SN could be found with false-negative rate of 2.6% (ie, tumor-positive LN was not detected by Lymphoseek) and a negative predictive value of 98% (28).

Sentinel node biopsy vs elective regional node dissection: Besides decreased morbidity, SNB offers other advantages over a neck dissection: i) Identification of aberrant draining patterns and "skip metastasis" (35–37); and ii) Improved (micro-) metastasis identification at (histo-)pathology; fewer SNs are harvested which are more meticulously evaluated by the pathologist, resulting in improved diagnostic accuracy (38,39). However, challenges remain: i) Local tracer injection is complicated in areas deep in the oral cavity; ii) Near-injection site SN identification can be challenging due to overshadowing of signal at the primary tumor signal (40,36,22); iii) Often multiple SNs are identified during surgery (22) increasing the risk of complications (41) (eg, nerve damage, lymphedema, scar tissue formation); iv) neck dissection after a positive SNB is complicated due to the presence of scar tissue; and v) A false-negative SNB occurs in 3 to 10% of subjects (ie, the SN is tumor-negative, but at follow-up metastasis in remaining regional LNs are found (42–45). Moreover, although LN 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 LN assessment can thus only be assessed in tumor types that are accessible for local injection**. This is significant because it influences standard of care (LN 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, the tracers used for SNB are not tumor-specific and thus SNB, similar to neck dissection, does not allow for intraoperative tumor-positive LN identification (21,46,47). In fact, **for both SNB and neck dissection (histo-)pathological evaluation is required to determine the presence of (micro-) metastasis and thus a method to intraoperatively identify metastatic LNs is lacking**. **Thus, HNSCC subjects would benefit from an alternative technique that, allows for intraoperative lymph node staging.**

We propose to evaluate the fluorescently-labelled anti-EGFR antibody, panitumumab-IRdye800, for its ability to intraoperatively identify sentinel and LN (micro-)metastasis. We propose to directly compare fluorescence imaging of panitumumab-IRDye800 imaging findings to that of SNB and neck dissection whereby we will use (histo-)pathology as gold standard for determining the true tumor-positive LNs.

Potential benefits of panitumumab-IRdye800 for metastatic LN identification over SNB:

1) Panitumumab-IRDye800 is a tumor-targeted tracer that can allow intraoperative identification of metastatic lymph nodes (for the other techniques pathology is required to confirm).
2) Panitumumab-IRDye800 is systemically injected, and thus can allow metastatic LN identification also at sites that are not-accessible for a SN tracer (ie, tumor in the hypopharynx).
3) Panitumumab-IRDye800 provides a tumor-specific approach whereas the SN tracer is not tumor-specific.
4) Panitumumab-IRDye800 can be injected several days before the operation and thus scheduling logistics would be improved (SN tracer is preferable injected the day of surgery)
5) If successful with the optical dye, similar studies could be conducted with a radiolabeled dye.

Fluorescent, tumor-targeted antibodies for intraoperative cancer detection: Clinical data has provided evidence that novel tumor-targeted antibodies can improve detection, and survival in a variety of cancer types (48). By fluorescently labelling these antibodies (eg, using the near-infrared fluorescent dye IRdye800) it was shown that the sensitivity and specificity of (microscopic) tumor detection in the operative setting could be significantly improved (49–52). Improved visualization can thus significantly improve surgical techniques and outcome.

The epidermal growth factor receptor (EGFR) is found overexpressed in 80 to 90% of subjects with HNSCC (53). Our group has shown that EGFR-based optical imaging can detect subclinical disease (non-palpable tumor) within the resection bed approximately 72 hours after systemic administration of a fluorescently labeled anti-EGFR antibody (54–56). The potential of optical imaging to guide surgical resection by capitalizing on the overexpression of EGFR has also been supported by the findings of other groups in a variety of solid tumors (57–59). Moreover we've shown the feasibility of real-time fluorescence imaging-based identification of HNSCC using the anti-EGFR antibody cetuximab-IRdye800 (49,60,61). In our phase 1 study, in all subjects, tumor tissue could be clearly discriminated from normal tissue, and a high fluorescence intensity strongly correlated with the presence of tumor (36,41). **Moreover, we showed that with another anti-EGFR antibody, cetuximab-IRdye800 we could identify metastatic LNs with a high 97.2% sensitivity; 92.7% specificity; 99.7% negative and 50.7% positive predictive value** (62). More recently we switched to panitumumab-IRDye800 – our phase 1 study (IRB-35064) evaluating the safety and dosing of panitumumab-IRDye800 in HNSCC is reaching completion. Preliminary results of this phase 1 study are in line with our previous clinical studies using cetuximab-IRdye800 **and suggests that we will be able to**

identify LNs via near-infrared fluorescence imaging of panitumumab-IRDye800 not detectable by conventional white light (Figure 2).

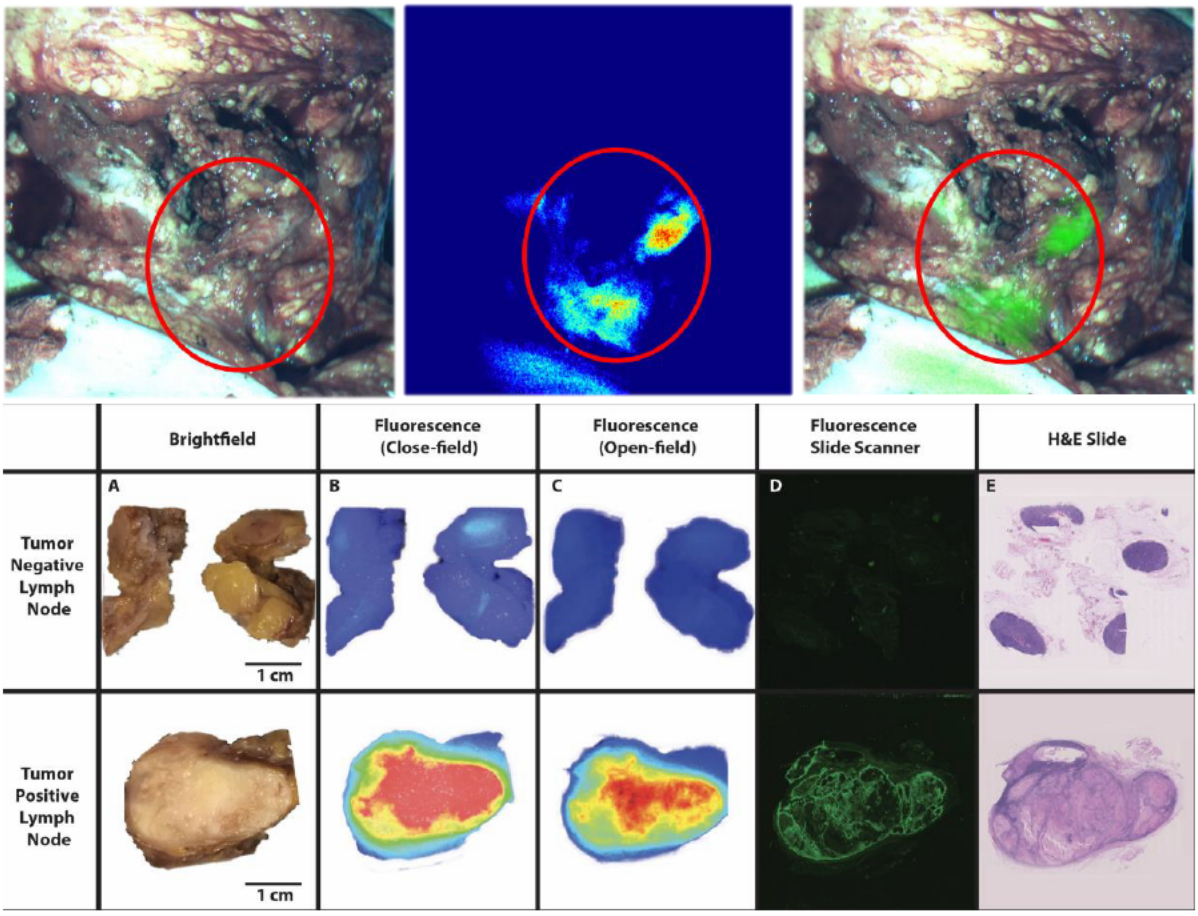


Figure 2: Near-infrared fluorescence imaging-based lymph node identification in a subject with HNSCC. Upper three panels: Brightfield (left); fluorescence (middle); and overlay (right) image showing a cluster of LNs in the neck. Lower panels: Evaluation of a formalin-fixed tumor-positive and tumor-negative LN illustrating differences in panitumumab-IRDye800 uptake.

Although EGFR is clearly a good target for HNSCC, **we chose to abandon cetuximab-IRDye800 and move forward with another near-infrared fluorescence labeled antibody, panitumumab-IRDye800, because:** **1)** Panitumumab is a fully humanized anti-EGFR-antibody resulting in fewer infusion reactions (cetuximab Grade 1-2 infusion reactions are as high as 30% (63,64); **2)** Panitumumab is a member of the IgG2 ubclass of IgG proteins in human serum, which have lower complement activation and low Fc receptor binding, and thus induces minimal inflammation and toxicity; **3)** Tumor-to-background ratios obtained with panitumumab-IRDye800 are similar, if not better, than with cetuximab-IRDye800 (65); and **4)** Panitumumab displays an improved safety profile; we have only seen <u>one</u> Grade 1 adverse event reported in 37 subjects that were infused with panitumumab-IRDye800 (IRB-35064) compared to <u>15 Grade</u> 1 adverse events in 12 subjects that were given cetuximab-IRDye800 (NCT01987375). In addition, in the phase 1 cetuximab-IRDye800 study, in 2 subjects the study drug was not given because of an infusion reaction to cetuximab (66,67).

2.2 Study Agents

The investigational agent for this study, panitumumab-IRDye800, is unapproved by FDA, and an Investigational New Drug application (IND) is required for this study. [Note: the antibody moiety of the investigational agent is approved by FDA as Vectibix (panitumumab)]. Lymphoseek is an FDA-approved agent for oral cavity SNB.

This protocol will be added to existing IND-119474 (IND Holder: Eben Rosenthal, MD).

2.3 Rationale

Overall impact of the proposed study on surgical oncology: Introduction of this technology to the operating room in the manner we propose would represent a paradigm shift in oncologic surgery as there are currently no techniques available that allow intraoperative detection of metastatic LNs. The use of optical imaging would be a significant improvement in real-time identification of cancer in LNs.

Our study would demonstrate important proof-of-principle and translate concepts that molecular biologists have used for more than a decade to identify and label cancer in real-time. At the same time it will allow us to evaluate the value the panitumumab-IRDye800 approach can have when compared to the current standards.

Not only can this have strong impact on subjects with HNSCC, the development of a cancer specific contrast agent of this type can also be applied to other solid tumors that express EGFR for which LN status of prognostic value. Furthermore, if the principle of targeted optical imaging is validated using EGFR, other therapeutic antibodies could be assessed for imaging potential. Successful completion of this study will thus have a lasting impact on the field of surgical oncology.

Our proposed study offers the potential to improve current standard of care in the following ways:

1. **Despite the variety of techniques available for metastatic LN detection, consensus is lacking** (46,68)
2. **Currently no intraoperative technique is available to directly identify tumor-positive LNs**: Panitumumab-IRDye800 has already shown to allow for real-time fluorescence imaging of the primary tumor (IRB-35064).
3. **Real-time feedback**: Presence of regional disease can be assessed intraoperatively.
4. **Minimally-invasive approach**: Presence of regional disease can be assessed intraoperatively, leading to fewer healthy LNs removed.
5. **Use in other cancer types**:
    - EGFR is highly-expressed in multiple cancer types, allowing application to other tumor types beyond HNSCC.
    - Our proposed technique can even be extended to tumors that are not accessible for superficial injections for SNB but for which LN status plays an important role, eg, colorectal cancer.

6. **High feasibility**: We have previously conducted dose-escalation phase 1 clinical trials using cetuximab-IRDye800 and panitumumab-IRDye800 in HNSCC ((69) and (65,68), respectivley).

2.4 Study Design

- The primary purpose for the protocol is **Image Guidance**. The primary objective of the study is to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck LNs of subjects with HNSCC.
- There are **2 cohorts**.
- The study is **open-label**. No masking is used.
- The study is **non-randomized**.
- The primary outcome is **number and location of LNs that are near-infrared fluorescent and that contain tumor as defined by (histo-)pathology**.

The study is an open-label study to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck LNs of subjects with HNSCC. Panitumumab-IRDye800-based findings will be compared to LN biopsy and neck dissection. A schematic overview of the clinical trial is given in Figure 1.

2.4.1. Number of subjects

A total of 20 subjects will be enrolled in our exploratory study.

Rationale for including cN0 and cN+ subjects: Based on NCCN guidelines, version 1 2018, SNB is justified in subjects with a T1 or T2 stage primary tumor and a cN0 neck that are at (high) risk for having LN metastasis. The strength of SNB lies in the identification of "skip lesions" (ie, metastatic spread to a LN outside the expected drainage area) and aberrant drainage patterns (ie, drainage to LNs that are outside the expected drainage template) (12-14). Including cN0 subjects will thus provide us with the unique opportunity to directly compare the performance of panitumumab-IRdye800 for metastatic LN identification to that of Lymphoseek and thus validate our approach against the reference standard.

Panitumumab-IRDye800 will be evaluated in subjects with any T stage and a cN+ neck to study how well panitumumab-IRDye800 can detect metastatic lesions in the LN(s).

2.4.2. Dosing

Subjects will be infused with 50 mg panitumumab-IRDye800 and 2 mCi Lymphoseek. Panitumumab-IRDye800 will be systemically infused 1 to 5 days prior to surgery, whereas 2 mCi Lymphoseek will be administered locally on the day before surgery (Table 1).

Table 1: Cohort structure

Cohort	Preoperative nodal stage	Number of subjects	Dose panitumumab-IRDye800	Dose Lymphoseek
1	T1 or T2 stage and node negative (ie, cN0)	10	50 mg	2 mCi
2	Any T stage and node positive (ie, cN+)	10	50 mg	none

2.4.2.1. Rationale for panitumumab-IRDye800 dosing

The dose we use in the current study is derived from the optimal dose as determined in Stanford IRB protocol IRB-35064 whereby we evaluated the safety and dosing of panitumumab-IRDye800 in HNSCC. In this study, we also investigated the necessity of an unlabeled-panitumumab loading dose. We found no significant differences in fluorescence intensities around the primary tumor site and similar TBRs when no loading dose was administered. Moreover, the 50 mg dose was found safe (no Grade 2 or greater toxicity reported) and easy to administer (unpublished data; see safety reports IRB-35064).

2.4.2.2. Safety Monitoring

Following our current IRB-35064, during and after the panitumumab-IRDye800 infusion, subjects will be monitored for allergic reactions. Safety measurements will be taken, including, as hemodynamic monitoring, ECGs, blood tests, and physical examination - see Section 9 for a specific list of measurements taken at the different timepoints (ie, screening, day of surgery).

If three or more participants of the study experience dose limiting toxicity (DLT), then the study will be stopped and the data reviewed. Dose-limiting toxicity (DLT) will be defined as any Grade 2 or greater toxicity that is considered possible, probably or definitely related to the study drug and considered clinically significant by the investigator, and occurs within 15 days of receiving treatment, which represents more than 4 half-lives of the drug. Restarting the study will require reassessment after discussion and approval by the Data Safety Monitoring Committee. Because panitumumab is an approved agent with a known DLT rate of 1%, we expect this study to further confirm the safety of conjugated panitumumab-IRDye800 at lower doses than the dose used in a therapeutic setting.

The known rate of severe infusion reactions of panitumumab is 1% (package insert, June 2017); therefore, we expect a DLT rate of approximately 1% or higher with panitumumab-IRDye800. Assuming the true DLT rate is as high as 5% and a significance level of 0.05, the probability of stopping early (three or more DLT in the first 12 subjects) is 0.002.

For Lymphoseek, no infusion reactions have been reported (see package insert) (28–30,32–34). The most common adverse reactions (incidence < 1%) are injection site irritation and pain (see Section 5.2.2.). Therefore, there is no implementation of additional safety measures warranted for this study.

2.4.2.3. Pharmacokinetic intervals

Clearance of panitumumab occurs through the binding in the liver and through the reticuloendothelial system as occurs with endogenous immunoglobulin. Because the

pharmacokinetics of single dose panitumumab are well known and the pharmacokinetics of panitumumab and panitumumab-IRDye800 are nearly the same in non-human primate studies (see toxicology section), only limited pharmacokinetics will be performed. Blood levels of panitumumab-IRDye800 and free IRDye800 will be monitored on Day 0 before infusion and approximately 1hour after end of infusion), on Day 1 as available, on the day of surgery (Day 1 to 5 post-infusion), Day 1 to 3 post-surgery as available, Day of discharge, and Day 30 post-infusion of the study drug.

2.4.3. Workflow of the Clinical Trial

Below we provide a concise summary of the clinical trial workflow with regard to the different steps that are taken upon receiving of written informed consent from the study subject.

2.4.3.1. Panitumumab-IRDye800 Infusion

Subjects will be infused with 50 mg panitumumab-IRDye800 1 to 5 days prior to surgery. Further details on the infusion can be found in Section 4.1 and 4.2.

2.4.3.2. Preoperative Sentinel Node Mapping

Currently, SNB of squamous cell carcinoma of the head and neck is not performed at Stanford. It is however in the NCCN guidelines (7) where it is advocated as an alternative to neck dissection for loco-regional staging of the neck in subjects with either T1 or T2 stage and N0 status. For the subject work-up we will follow literature references on Lymphoseek in HNSCC (28), and the standard operating protocol the Division of Nuclear Medicine has in place for SNB of melanoma using Lymphoseek.

Current Procedural Terminology (CPT) codes are available for injection of the radiopharmaceutical Lymphoseek and (sentinel) lymph node mapping (38792 and 78195, respectively) in oral cavity cancer (ICD-10 codes C01-C06.9). The costs of the SNB procedure will therefore be billed to the subject's medical insurance.

Lymphoseek will be locally administrated the day before surgery. Peritumorally, 4 x 0.1 mL of Lymphoseek will be administrated. A total dose of maximally 2 mCi will be administered (28). Further details on Lymphoseek administration and radiation exposure are described in 4.1 and 4.3, respectively.

Preoperative SN mapping in subjects with HNSCC will be performed according to the protocol the Division of Nuclear Medicine has for Lymphoseek-based SN mapping in melanoma. Dynamic images will be acquired in anterior projection over the head and neck starting immediately after the injections have been completed. Lymphoscintigraphic imaging is performed at a 1 minute/frame for 30 minutes using a 128x128 matrix, followed by static spot views with/without a transmission source. Single photon emission computed tomography combined with computed tomography (SPECT/CT) imaging of the head and neck will follow the planar dynamic imaging. Acquisition parameters for a multi-detector system are 3-degree angular sampling, 128 x 128 matrix, 360-degree rotation and 20-30 sec per stop.

Following completion of image acquisition the Nuclear Medicine physician will review the images to determine the direction of the lymphatic flow and the potential accumulation in a sentinel LN. Using SPECT/CT images, he/she will determine the number and location of the SNs.

Rationale for SPECT/CT imaging: The value of SPECT/CT lies in the anatomical localization of the spot views seen on the dynamic planar images. Moreover, SPECT/CT may help to localize foci of abnormal Lymphoseek accumulation more accurately than planar imaging or SPECT alone.

Radiation risks associated with SPECT/CT imaging: It has been recommended that when SPECT/CT imaging is performed for anatomical correlation with the functional study, a low-dose CT scan is recommended. The radiation dose from low-dose CT scans will be approximately 0.6 mSv (70). This value is up to 80-85% lower than the radiation dose received when performing a diagnostic CT. As reported by Montes, *et al*, the main radiation risk for these low doses, in which stochastic effects predominate, is an increased probability of cancer induction – ranging 0.003 to 0.010% (71).

2.4.3.3. Intraoperative Imaging Protocol

On the day of surgery, 1 to 5 days post-infusion of panitumumab-IRDye800, intraoperative fluorescence imaging and gamma tracing will be performed.

Optical imaging devices to be used: Imaging with the optical imaging devices will be performed intraoperatively in situ and on the back table (see Appendix B for a list of the imaging devices). Additional ex vivo devices will also be used to image the specimens on the back table (see Appendix B for a list of the imaging devices).

All in vivo devices are FDA approved for measuring intraoperative blood flow after indocyanine green (ICG) injection. Because IRDye800 and ICG have overlapping excitation and emission spectra, each device can also be used for imaging subclinical disease based on our preclinical studies in head and neck cancer (see Appendix B for a list of the imaging devices).

Sentinel node biopsy (stage T1/2, cN0)

Step 1) *In situ* gamma tracing and fluorescence imaging of the sentinel nodes:

1. Prior to opening the skin, the surgeon will screen the neck for the presence of radioactive node(s) (ie, SNs) by sweeping the gamma probe over the intact neck. If any radioactive nodes are detected, the location of detected radioactive nodes will be marked on the skin using indelible ink and a still image of the marked will be collected for documentation.
2. After opening the skin, a photo of the neck will be taken for documentation. Hereafter the surgeon will perform fluorescence imaging of the neck to evaluate if there are any fluorescent nodes visible. Fluorescent nodes will be marked on the photo of the neck. Video and still images of the fluorescence imaging procedure will be collected.
3. Hereafter, as per standard of care, the surgeon will perform SNB using the gamma probe. For this, the surgeon will evaluate the neck with the gamma probe for the presence of radioactive nodes. Upon localizing of radioactive nodes, the count rate of the node will be determined. Then, just prior to the resection of the radioactive node, *in situ* fluorescence imaging will be performed to evaluate the fluorescence-based visibility of the node. For each radioactive node, the respective *in situ* location will be documented (level Ia, Ib, IIa, IIb, III, IV or V) as well as the radioactive status (count rate) and fluorescence status (yes/no). Hereafter the node will be harvested by the surgeon. This process will be repeated until no radioactive nodes are detected anymore *in situ*.

Step 2) Wound bed imaging:

As per standard of care SNB, upon completion of SNB, the neck will be screened once more with the gamma probe as such to determine the presence of any remaining radioactivity (ie, a radioactive signal detected > 10% of the background that correlates with any substrate (node). Areas in the wound bed determined radioactive-positive will be surgically re-evaluated, and, upon checking the fluorescence-status, removed, if deemed necessary by the surgeon. If any nodes are found, gamma tracing will be performed to determine the radioactive status.

Step 3) Pathological imaging:

The fluorescence and/or radioactive status of all excised nodes will be confirmed ex vivo on the back table using both a closed-field imaging device and an open-field device as well as the gamma probe, respectively. Hereafter all excised nodal specimens will be sent for (histo-)pathology. After the tissue has been processed and evaluated by the pathologist, imaging findings will be compared to gold-standard histological-assessment performed by the pathologist (including a hematoxylin and eosin (H&E) staining) to determine the correlation between fluorescence, radioactivity and the presence/absence of tumor tissue in the LN(s). Also findings will be compared to preoperative lymphoscintigraphy and SPECT/CT imaging (ie, correlation between preoperative imaging findings and intraoperative imaging findings).

Neck dissection only (T1-4, cN+)

After the neck has been opened by the surgeon, it will be inspected by the surgeon for the presence of suspicious nodes. Real-time fluorescence imaging of the LNs in the neck will then be performed at 4 time points:

Step 1) *In situ* imaging: After having opened the skin and exposure of the neck LNs, *in situ* fluorescence imaging will be performed to evaluate the fluorescence-based visibility of the LNs. For each fluorescent LN, the respective location will be documented (level Ia, Ib, IIa, IIb, III, IV or V) after which it will be harvested by the surgeon. This process will be repeated until no fluorescent LNs are seen *in situ*. Hereafter, the standard of care completion neck dissection will be performed.

Step 2) E*x vivo* imaging: The neck dissection specimen will be evaluated for the presence of fluorescent LNs using both a closed-field imaging device and an open-field device. All additional fluorescent LNs will be identified, documented and collected separately; we acknowledge that the penetration depth of the fluorescence signal is limited and therefore deeper located LNs might not be detectable *in situ* (under step 1), but after having taken out the specimen and having "flipped" the specimen this might be possible.

Step 3) Wound bed imaging: This will be conducted for any remaining fluorescence (ie, any remaining fluorescent LNs). Areas in the wound bed determined fluorescence-positive will be surgically re-evaluated, and, if deemed necessary by the surgeon, removed.

Step 4) Pathological imaging: All excised nodal specimens will be imaged once more in the closed-field device to confirm that all fluorescent LNs have been removed from the neck dissection specimen. If not, the number and location of remainder fluorescent LNs will be documented after which they will be harvested; we will use the image taken under step 2 for reference as such to make sure that we did not create any confounding factor due to tissue

preparation to harvest nodes at step 2. Hereafter all excised nodal specimens will be sent for (histo-)pathology. After the tissue has been processed and evaluated by the pathologist, fluorescence imaging-based findings will be compared to gold-standard histological-assessment performed by the pathologist (including a hematoxylin and eosin (H&E) staining) to determine the correlation between fluorescence and the presence/absence of tumor tissue in the LN(s).

2.4.3.4. Laboratory Imaging Protocol

Once the LNs have been removed from the subject and sent for pathology review, additional imaging will be performed with non-significant risk devices (Pearl and Odyssey imaging systems – LICOR Biosciences, Lincoln, NE) of pathological specimen(s) using non-invasive imaging modalities that do not expose the subject to radiation or other interventions, or alter the standard pathological processing of the tissues (see Appendix B for a list of the imaging devices). Normal waste tissues will also be imaged. The research imaging modalities will not be used in the patient-care setting. Imaging will be done in collaboration with the Department of Pathology to ensure preservation of tumor-related information. Data from the research use of the device will not be used for diagnosis or other medical decision-making.

2.4.3.5. Pathological Correlates

Tissue specimens will be collected by pathology after which they will be formalin-fixed and paraffin-embedded according to standard procedures.

Specifically, for the LN specimens we will investigate the following:

To determine biological characteristics associated with fluorescence intensity measurements, formalin-fixed paraffin-embedded blocks from the collected specimens will be obtained from pathology after routine analysis and imaged on the Pearl and Odyssey as previously described in preclinical models (72,73). This will allow high-resolution evaluation of the location of the fluorescence signal in the tissue. Subsequently 5 micron sections will be cut for histological analysis of: 1) Size of the epithelial tumor compartment in percent of total; 2) Tumor characteristics (ie, size, proliferation); and 3) EGFR. Methods have been previously described by us using *in vivo* models (74–78).

We will then determine if these characteristics are associated with fluorescence (yes/no) from the Pearl and Odyssey imaging systems. We will use the two-sided Fisher's exact test to determine the level of association between tumor characteristics (ie, size, proliferation) and EGFR status and fluorescence (yes/no). Likewise, we will test the association between the size of the epithelial tumor compartment and fluorescence (yes/no) with the two-sided Wilcoxon rank sum test.

To determine where panitumumab-IRDye800 localizes in the fluorescent LNs, 5 micron sections will be cut and undergo immunohistochemistry for localization of various immune cells, including B and T-cells, macrophages and dendritic cells. Immunohistochemistry findings will then be correlated with fluorescence intensities measured on the Pearl and Odyssey imaging systems. We expect that panitumumab-IRDye800 both specifically and non-specifically occurs in the LNs. Specific uptake in the LNs will occur if metastasis are present in the nodes. Non-specific uptake of panitumumab-IRDye800 in the LNs can occur by a mechanism similar to the principle of SNs mapping, ie, due to accumulation of tracer at the injection site, overflow from the tumor to the LNs will occur where this tracer will get trapped in immune cells (79).

The two-sided Fisher's exact test will be used to determine the level of association between each of these characteristics and panitumumab-IRDye800 fluorescence (yes/no).

2.4.3.6. Statistical Analysis

The statistical analysis, including sample size justification and power considerations are given in Section 12.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

a. Biopsy confirmed diagnosis of squamous cell carcinoma of the head and neck

b. Subjects with recurrent disease or a new primary will be allowed

c. Planned standard of care surgery with curative intent for squamous cell carcinoma

d. *** SNB only cohort:** Subjects diagnosed with a T1-T2 stage tumor, any subsite within the head and neck that is amenable to local sentinel node tracer injection and are scheduled to undergo surgical resection of the tumor, including a sentinel node biopsy

e. *** Neck dissection only cohort:** Subjects diagnosed with any T stage, any subsite within the head and neck that are scheduled to undergo surgical resection, including a (modified) neck dissection

f. Age ≥ 19 years

g. Karnofsky performance status of at least 70% or ECOG/Zubrod level 1

h. Have acceptable hematologic status, coagulation status, kidney function, and liver function including the following clinical results:

   i. Hemoglobin ≥ 9 gm/dL

   ii. White blood cell count > 3000/mm^3

   iii. Platelet count ≥ 100,000/mm^3

   iv. Serum creatinine ≤ 1.5 times upper reference range

**Only one criterion to be checked for each participant – either "d" or "e".*

3.2 Exclusion Criteria

a. -Myocardial infarction (MI); cerebrovascular accident (CVA); uncontrolled congestive heart failure (CHF); significant liver disease; or unstable angina within 6 months prior to enrollment

b. History of infusion reactions to monoclonal antibody therapies

c. Pregnant or breastfeeding

d. Magnesium or potassium lower than the normal institutional values

e. Subjects receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents

f. Subjects with a history or evidence of interstitial pneumonitis or pulmonary fibrosis

g. Hypersensitivity to dextran and/or modified form thereof

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 (ie, SLN0028-001, -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 18 months from the time the study opens to accrual; we anticipate enrolling 1 to 2 subjects per month from the start of the study.

Study Completion:

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

The anticipated study start date: December 2018 – January 2019

The anticipated study completion date: December 2020

4. TREATMENT PLAN

4.1 Treatment Schedule

Subjects will receive intravenous panitumumab-IRDye800 (50 mg) 1 to 5 days prior to surgery. Then on the day before surgery, subjects in Cohort 1 will receive a local injection with 2 mCi Lymphoseek followed by lymphoscintigraphy and SPECT/CT imaging to determine the number and location of LNs. In the operation theatre, using a combination of fluorescence imaging and radio-tracing, the LNs of the neck will be evaluated and scored (Section 2.4, Figure 1).

4.2 Administration of panitumumab-IRDye800

Administration of panitumumab-IRDye800 will be performed consistent with institutional policy for administration of panitumumab. The solution will be diluted to a total volume of 100mL using 0.9% sodium chloride injection, USP. Dosage level will be 50 mg panitumumab-IRDye800 (< 1/12 of a therapeutic dose of panitumumab). Administration of panitumumab-IRDye800 will occur by IV over 15 (± 5) minutes. Subjects will be observed for 30 (± 10) minutes after administration. Additional treatment for infusion reactions and electrolyte imbalances may be given following institutional policy.

Premedication will be delivered prior to the infusion for each subject at the discretion of the treating physician according to routine institutional protocols for delivery of panitumumab.

Treatment will be given in an infusion facility that routinely administers experimental chemotherapeutic infusions and is equipped with emergency equipment and medication along with medical personnel in the event that the subject experiences a severe reaction.

The infusion(s) will be stopped if deemed necessary with the onset of any significant signs of reaction. Steroids and other supportive medications may be administered and infusion resumed at the discretion of the treating physician. If a subject experiences a serious reaction that prevents completing the study infusion(s), the subject will be observed; but will not complete their study infusion(s). Any subject who does not receive the full panitumumab-IRDye800 dose will be considered withdrawn from the study and not count towards cohort accrual.

With a single dose at the levels proposed the half-life is expected to be approximately 2.3 days or less. Clearance of panitumumab occurs through the binding in the liver and through the reticuloendothelial system as occurs with endogenous immunoglobulin.

4.3 Administration of Lymphoseek (^{99m}Tc-Tilmanocept)

Lymphoseek is supplied as a unit dose from Cardinal Health.

The radiopharmaceutical Lymphoseek will be administered locally at the Division of Nuclear Medicine. A total of 4 peri-tumoral injections of 0.1 mL of Lymphoseek will be given with a total dose of 2 mCi Lymphoseek injected. Per physicians discretion, local anesthesia may be used (ie, viscous lidocaine, lidocaine spray).

Radiation risk Lymphoseek: Exposure to radiation causes both direct DNA damage (single-strand or double-strand DNA breaks) via ionization (energy is transferred to electrons and the atom loses an electron becoming ionized) and indirect damage via the formation of free radicals. Adhere to As Low as Reasonably Achievable (ALARA) principles (dose recommendations) and ensure safe handling (time, distance, and shielding) to minimize the risk for excessive radiation exposure to either patients or health care workers.

Technetium-99m decays by isomeric transition with a physical half-life of approximately 6 hours. The estimated local radiation dose varies greatly on the time to surgery and the adminstered dose as well as the injected location (27). The recommended dose of Lymphoseek is 0.5 mCi (18.5 MBq) as a radioactivity dose and 50 mcg as a mass dose for a 1-day protocol (i.e., surgery is performed within 15 hours) and 2 mCi (74 MBq) for a 2-day protocol (i.e., surgery is performed the day after injection of Lymphoseek).

No specific **adsorbed radiation dose for Lymphoseek** has been reported **in patients** with oral cavity carcinoma, however, for breast cancer and melanoma adsorbed radiation doses are reported in the package insert of Lymphoseek when a dose of 0.5 mCi is injected (Table 2). The effective dose equivalent for males and females are 0.20 and 0.25 mSv in melanoma and 0.30 and 0.33 mSv in breast cancer, respectively. These doses are in line with previous SNB reports from amongst others Memorial Sloan Kettering Cancer Center (80). As for the absorbed radiation dose for the surgical staff in the operation room, this is < 0.0001 mSv per operation with a maximum dose of < 0.0002 mSv to the surgeon (27) – these doses are further minimized when the surgery is performed > 24 hours after radiotracer administration. Monitoring of the operation room personnel and additional shielding is therefore not required.

For a 2 mCi administrated Lymphoseek dose, using Table 2 as reference, we estimated the effective dose equivalent for males and females being 0.810 and 1.05 mSv in melanoma and 1.18 and 1.32 mSv in breast cancer, respectively. **Extrapolating this to our HNSCC subjects, the total amount of radiation from one SNB procedure using Lymphoseek will then range 1.41 to 1.78 mSv in males and 1.65 to 1.97 mSv in females – this includes a 0.6 mSv dose that the subject will receive when the low-dose CT is acquired. This is approximately 4% of the total radiation a person will receive in one year from the environment.**

Table 2: Estimated absorbed radiation dose from 0.5 mCi Lymphoseek in subjects with breast cancer and melanoma

Target Organ	Breast Cancer[a] mGy (rad)	Melanoma[b] mGy (rad)
brain	0.003 (0.0003)	0.0927 (0.0093)
breast (injection site)	1.659 (0.1659)	0.7903 (0.079)
gall bladder wall	0.0349 (0.0035)	0.0712 (0.0071)
lower large intestine wall	0.0123 (0.0012)	0.057 (0.0057)
small intestine	0.0101 (0.001)	0.0594 (0.0059)
stomach	0.0184 (0.0018)	0.0562 (0.0056)
upper large intestine wall	0.0125 (0.0012)	0.0582 (0.0058)
kidney	0.1863 (0.0186)	0.278 (0.0278)
liver	0.0324 (0.0032)	0.0929 (0.0093)
lungs	0.0374 (0.0037)	0.0599 (0.006)
muscle	0.0092 (0.0009)	0.0451 (0.0045)
ovaries	0.187 (0.0187)	0.2991 (0.0299)
red marrow	0.0127 (0.0013)	0.0507 (0.0051)
bone	0.0177 (0.0018)	0.0878 (0.0088)
spleen	0.0285 (0.0029)	0.0598 (0.006)
testes	0.0501 (0.005)	0.1043 (0.0104)
thymus	0.1168 (0.0117)	0.0577 (0.0058)
thyroid	0.088 (0.0088)	0.0464 (0.0046)
urinary bladder	0.0586 (0.0059)	0.1401 (0.014)
total body	0.0195 (0.0019)	0.0547 (0.0055)
Effective Dose Equivalent	**microSv**	**microSv**
males	296	202.4
females	330.2	251.1

[a] Calculated from data of 18 patients with breast cancer who received four peritumoral injections of 4 mcg, 20 mcg, and 100 mcg doses of Lymphoseek.

[b] Calculated from data of 18 patients with melanoma who received four intradermal injections of 20 mcg, 100 mcg, and 200 mcg doses of Lymphoseek. Due to the differences in injection sites among patients with melanoma, the injection site was assumed to be the breast for the purposes of this calculation, as it represents the nearest anatomical construct for the skin from the anatomical sites appropriately included in the estimates.

4.4 Definition of Dose-Limiting Toxicity (DLT)

AEs will be graded according to the CTCAE v5.0 (http://evs.nci.nih.gov/ftp1/CTAE/about.html).

Dose-limiting toxicity (DLT) will be defined as any Grade 2 or greater toxicity that is ALL of the following:

- Determined by the investigator to be possible, probably or definitely-related to panitumumab-IRDye800 or Lymphoseek
- Considered by the Investigator to be clinically significant for subjects who received panitumumab-IRDye800 or Lymphoseek. 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
- Occurred within 15 days of receiving treatment with panitumumab-IRDye800 or Lymphoseek

The Stanford Data Safety Monitoring Committee (DSMC) will be notified of all DLTs. The DSMC will evaluate safety data from all subjects through Day of discharge. If 3 or more subjects experience a DLT, then study enrollment will be halted pending DSMC review.

4.5 Surgical Imaging

Lymphadenectomy specimen assessment. Please see Section 2.4 for more detail. The neck will be imaged using near-infrared fluorescence and white light optical imaging systems and gamma tracing will be performed (Cohort 1 only). Imaging and tracing will be performed prior to lymphadenectomy, and after lymphadenectomy. Subsequently, the lymphadenectomy specimens will be imaged on a 'back table' prior to sending the specimens to pathology. The number and location of the fluorescent, and/or radioactive LNs will be documented.

The imaging information obtained will not be used for diagnosis or any other medical decision-making. Added surgical time for these images is expected to be minimal since these the images can be recorded for later review as still and video images. Once the specimens have been delivered to pathology, additional imaging will be performed as described above in Section 2.4.

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 from the day of admission for standard of care surgery until the day of discharge will not be collected, unless given for a reportable adverse event or reportable serious adverse event (See AE section for AE definition).

Premedication and supportive care for infusion reactions and/or electrolyte imbalances may be administered per institutional policies at the discretion of the treating physician.

4.7 Criteria for Removal from Study

Treatment will be stopped and subjects will be removed from study due to unacceptable AEs, or withdrawal of consent. Any subject who does not receive the panitumumab-IRDye800 will be considered withdrawn from the study and will not count towards enrollment goals.

Subjects will be considered off study once they have completed the 30-day safety observation period.

4.8 Alternatives and Risk Protection / Mitigation

Alternatives

- This study is in the setting of tumor and LN node excision of suspected squamous cell carcinoma, and the experimental component is infusion of panitumumab-IRDye800, and Lymphoseek with planar and SPECT/CT imaging, which may help the surgeon visualize LN status and location. Additionally, using a handheld gamma counter post-injection may also help the surgeons to locate nodes with Lymphoseek uptake. Both approaches have been shown to decrease overall time in surgery. The surgery itself is considered regular medical care. Therefore, the alternative to participating in this study is to have the surgery without panitumumab-IRDye800 and/or Lymphoseek administration.

Procedures to protect against and minimize potential risks:

- This study is designed enroll appropriate subject populations (see Section 3.1 Inclusion Criteria, and Section 3.2 Exclusion Criteria).
- Subjects will be monitored regularly for AEs by the investigators and the study team after investigational panitumumab-IRDye800 and surgery, through 30 days (± 7 days) post-panitumumab-IRDye800 infusion.
- 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 Drug

5.1.1. Panitumumab-IRDye800

Unlabeled Panitumumab

Panitumumab (PLS-125147, Vectibix) is a fully-humanized IgG_2 monoclonal antibody that is FDA-approved therapeutic antibody targeting the epidermal growth factor receptor (EGFR) and is indicated as a single agent for the treatment of metastatic colorectal carcinoma with disease progression. It is also approved in Canada and the European Union.

FDA Summary Basis of Approval excerpts are provided and summarized below. Panitumumab was evaluated for pharmacologic activity in preclinical rodent studies and for toxicity and pharmacokinetics in nude mice and cynomolgus monkeys. Tissue binding studies demonstrated that it bound with moderate to strong intensity to surface expressed EGFR in samples of both

human and cynomolgus monkey skin, tonsil, breast, and prostate, and in the genitourinary tract epithelium of monkeys.

In preclinical xenografts rodent studies, it was found that combination therapy with Panitumumab and chemotherapy or other anti-tumor treatments resulted in additive benefit. Pharmacokinetic profiles of Panitumumab in cynomolgus monkeys following systemic administration of single doses of 7.5, 15, 30, or 60 mg/kg showed linear, dose-related increases in C_{max} and dose-related decreases in clearance with a concomitant increase in the half-life. Steady state of the drug could be achieved following repetitive dosing approximately 5 to 6 doses of panitumumab. The half-life of panitumumab after single administration depends on the dose. It is known that when panitumumab is administered to humans as a single dose between 0.5 mg/kg and 1.5 mg/kg the half-life is 0.8 days; at 2.0 mg/kg the half-life is 2.3 days; and at 6.0 mg/kg the half-life 7.3 days (FDA Drug Approval Package, Clinical Pharmacology Biopharmaceutics Review, application 125147).

Panitumumab's therapeutic activity occurs by binding to the tumor cell expressed EGFR which 1) competitively inhibits the binding of its normal ligands including epidermal growth factor and transforming growth factor-alpha, which are implicated in tumor growth, and 2) stimulates receptor internalization, leading to a reduction of EGFR signaling. Over-expression of EGFR is also detected in many cancer types including pancreatic, head and neck, lung, colon and brain.

Toxicity profiles associated with administration of panitumumab are known. Severe dermatologic and gastrointestinal toxicities were noted after repeated dosing in cynomolgus monkeys who received weekly treatment at therapeutic dosing: 7.5; 15; 30; or 60 mg/kg panitumumab for 4; 13 or 26 weeks. These doses correspond to approximately 1.25 to 10-fold greater than the therapeutic human dose of 6 mg/kg. The dermatologic toxicities associated with Panitumumab treatment were observed both in humans and preclinical toxicology studies (cynomolgus monkeys) following repeat administration and were not associated with single-dose administration. In clinical trials the dermatologic toxicities were generally Grade 2 in severity, with approximately 12% of subjects (95/789) reporting Grade 3 or higher skin changes. The package insert for panitumumab describes other toxicities associated with repeat administration including: electrolyte disturbances (hypomagnesemia and hypocalcemia), pulmonary fibrosis and ocular toxicities. Non-dose related toxicities include infusion reaction of approximately 4% (package insert). During clinical trials, no subjects had life-threatening or fatal infusion reaction to Panitumumab with severe events occurring in 1 % (NCI CTC grade 3 to 4). Most of the potential infusion reactions were mild in intensity and did not require treatment or interruption of panitumumab dosing. For additional toxicity information please see the package insert.

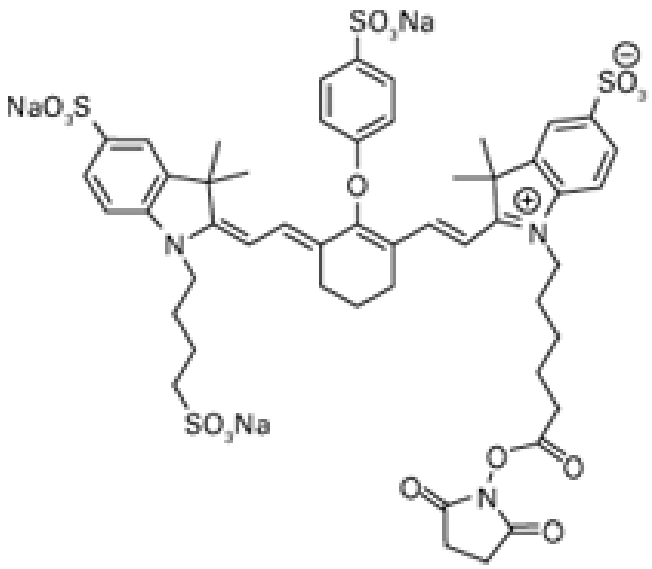
IRDye800CW

IRDye800CW N-hydroxysuccinimide (NHS) ester infrared dye (LiCor, Lincoln, NE) 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 Phosphate Buffered Saline (PBS) has an absorbance maximum of 773 nm and an emission maximum of 792 nm. IRDye800CW is a near infrared fluorescent imaging dye that can be easily bound to proteins through the NHS Ester group (Figure 3) and has emission and absorption wavelengths as described in Figure 4. Much of the information around IRDye800 remains proprietary, however, LiCor has filed a

master drug file with FDA (MF 25167) and produces the dye under cGMP conditions with appropriate documentation.

IRDye800CW has not been formally tested in humans and is not FDA-approved.

IRDye800CW has been assessed in rodents at 20 mg/kg without noticeable toxicity (81). This dose is several hundred times the proposed dose in the current trial. Although there are currently no publications available to demonstrate use of bioconjugated IRDye800, bevacizumab (Avastin) conjugated to IRDye800 is currently under investigation in Europe (http://clinicaltrials.gov/ct2/show/NCT01508572), but has not been tested in the US in any form that we are aware of, and is not approved for any indication. There have been no AEs associated with the administration of the bevacizumab-IDye800 (personal communication with Principal Investigator Dr van Dam).



IRDye® 800CW NHS Ester

$C_{50}H_{54}N_3Na_3O_{17}S_4$

Exact Mass: 1165.20291

Mol. Wt.: 1166.20297

Figure 3. IRDye800CW NHS-ester. Molecular formula and molecular characteristics

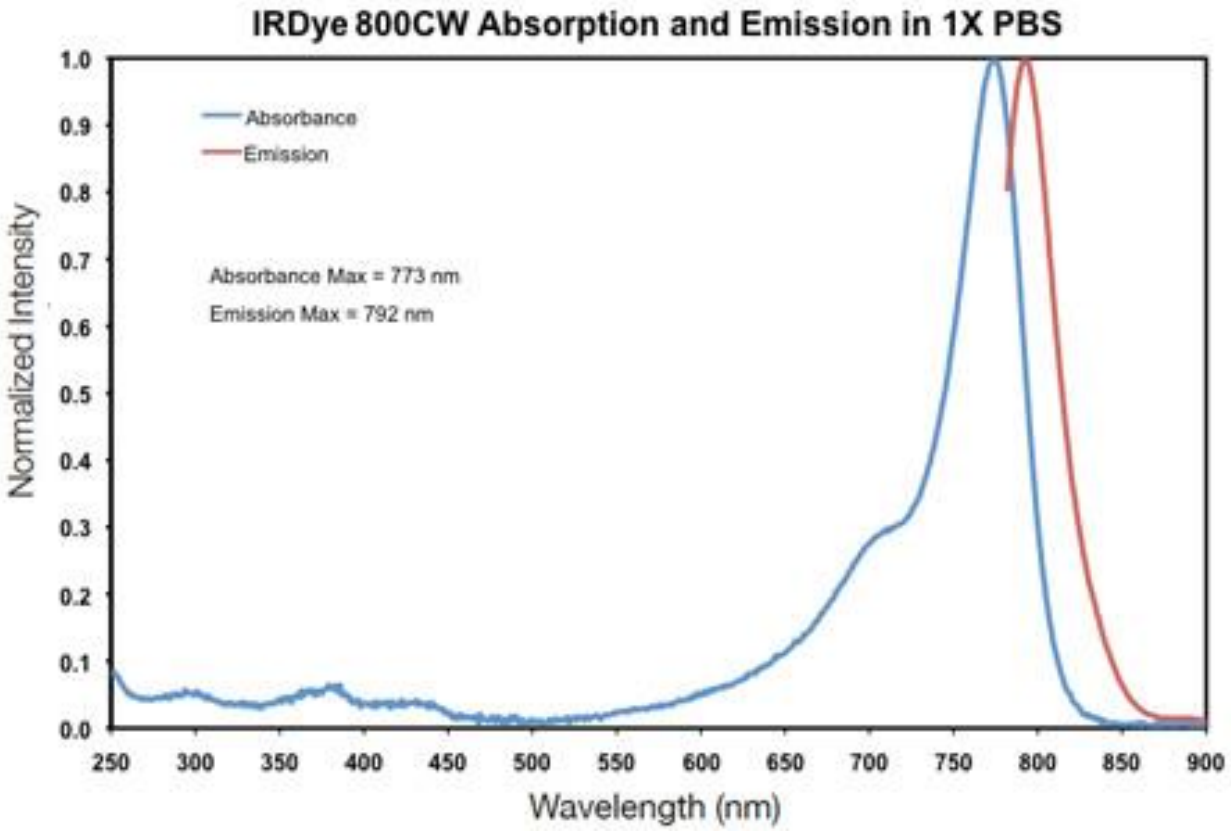


Figure 4. Absorption and emission spectra of IRDye800. Peak absorbance occurs at 773 nm and the peak emission is detected at 792 nm.

Panitumumab-IRDye800

Panitumumab-IRDye800 is produced by mixing panitumumab and IRDye800 in PBS at pH 8.5 and allowing the 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 was manufactured from commercially-sourced panitumumab and cGMP IRDye® 800CW N-hydroxysuccinimide (NHS) ester infrared dye (LI-COR Biosciences) at the GMP biologics facility at LI-COR Biosciences Inc. Formal testing of panitumumab-IRDye800 for stability, sterility and antigen specificity is contained within the IND 119474 CMC section according to an FDA approved process. The manufacturing and quality processes are documented elsewhere.

5.1.2. Lymphoseek

Lymphoseek is an FDA approved agent for lymphatic mapping and guiding SNB procedures. The details we've listed below are copied from the package insert of Lymphoseek.

The active ingredient in Lymphoseek, a radiodiagnostic agent, is ^{99m}Tc-tilmanocept (Figure 5).

Chemically, ^{99m}Tc-tilmanocept consists of Technetium-99m, dextran 3-[(2-aminoethyl)thio]propyl 17-carboxy-10,13,16- tris(carboxymethyl)-8-oxo-4-thia-7,10,13,16 tetraazaheptadec-1-yl 3-[[2-[[1-imino-2-(D mannopyranosylthio)ethyl]amino]ethyl]thio]propyl ether complexes.

The molecular formula of ^{99m}Tc-tilmanocept is $[C_6H_{10}O_5]_n.(C_{19}H_{28}N_4O_9S^{99m}Tc)_b.(C_{13}H_{24}N_2O_5S_2)_c.(C_5H_{11}NS)_a$. It contains 3-8 conjugated DTPA (diethylenetriaminepentaacetic acid) molecules (b); 12-20 conjugated mannose molecules (c) with 0-17 amine side chains (a) remaining free.

The calculated average molecular weight of tilmanocept ranges from 15,281 to 23,454 g/mol.

Mechanism of action: Lymphoseek is a macromolecule consisting of multiple units of DTPA and mannose, each covalently attached to a 10kDa dextran backbone. The mannose acts as a ligand for the mannose binding receptor (CD206), DTPA serves as the chelating agent for labeling with Technetium-99m.

Lymphoseek accumulates in lymphatic tissue and selectively binds to the mannose binding receptor located on the surface of macrophages and dendritic cells residing in the lymphatic tissue.

Pharmacodynamics: In in vitro studies, ^{99m}Tc-tilmanocept exhibited binding to human mannose binding receptors with a primary binding site affinity of $K_d = 2.76 \times 10^{-11}$ M.

In clinical studies, ^{99m}Tc-tilmanocept has been detectable in LNs within 10 minutes and up to 30 hours after injection.

Pharmacokinetics: In dose-ranging clinical studies, injection site clearance rates were similar across all Lymphoseek doses (4 to 200 mcg) with a mean elimination rate constant in the range of 0.222 to 0.396/hr, resulting in a drug half-life at the injection site of 1.8 to 3.1 hours.

The amount of the accumulated radioactive dose in the liver, kidney, and bladder reached a maximum 1 hour post administration of Lymphoseek and was approximately 1% to 2% of the injected dose in each tissue.

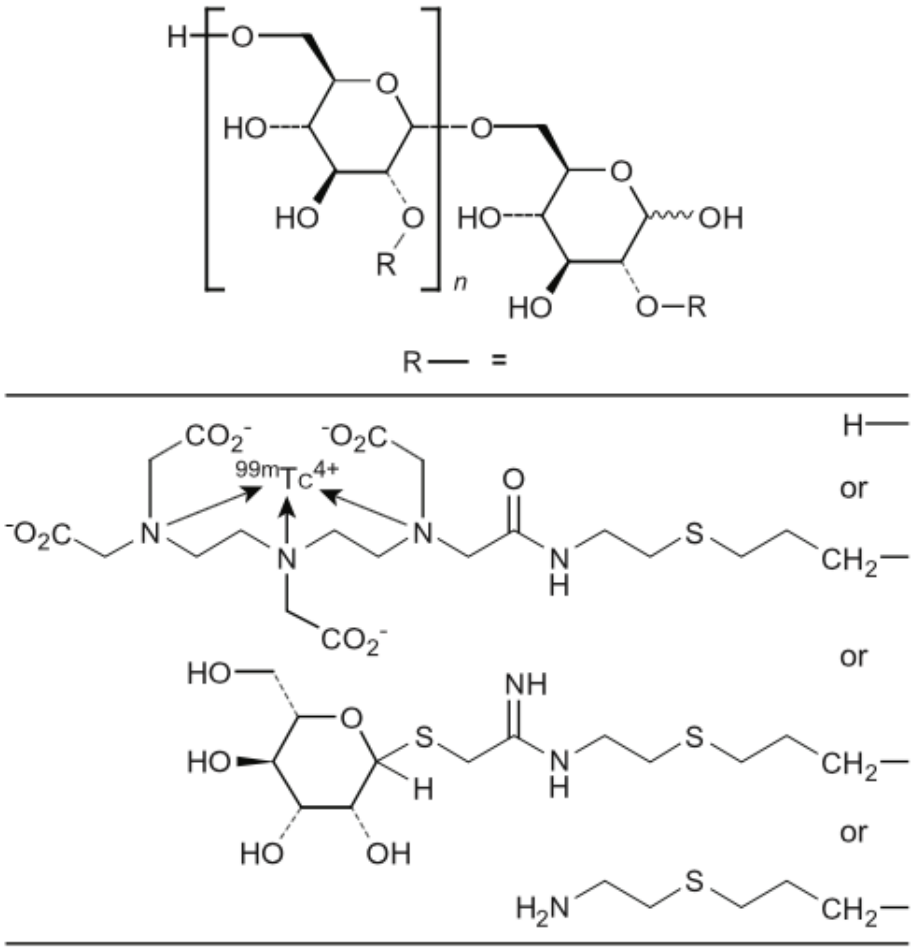


Figure 5: Chemical structure of Lymphoseek before (upper panel) and after (lower panel) labeling with Technetium-99m.

5.2 Expected Toxicities

5.2.1 Panitumumab – panitumumab-IRDye800

With the exception of infusion reactions and allergic responses, the 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, we anticipate the primary adverse events will be related to dose independent reactions including infusion reaction or allergic response.

In our current head and neck study using panitumumab-IRDye800, among the 55 subjects we have treated, we have only observed one possibly related grade 1 adverse event (elevated QTc) and no infusion reactions or DLTs.

5.2.1.1 Dermatologic and Soft Tissue Toxicity (Black Box Warning)

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.

5.2.1.2 Infusion reaction

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 4%, with severe reactions (anaphylaxis) reported in 1% of subjects (package insert). Serious infusion reactions—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.

5.2.1.3 Cardiac Toxicity

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 subjects at risk for arrhythmias, see exclusion criteria. Screening ECG will be obtained as part of the study. We reference the full toxicology report for cetuximab-IRDye800 (IND 115706) recently submitted to the FDA.

5.2.1.4 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.

5.2.1.5 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 when used as anti-cancer treatment. For this study, electrolytes will be monitored for 30 days after administration of panitumumab-IRDye800. Replete electrolytes as necessary using standard infusion guidelines.

5.2.1.6 Photosensitivity

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

5.2.1.7 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.

5.2.1.8 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 (81), and it has similar chemical structure as indocyanine green (which has been delivered to humans in gram quantities for decades without significant toxicity).

5.2.2 Lymphoseek (^{99m}Tc-Tilmanocept)

Below we provide a summary of the toxicity results that are mentioned in the Lymphoseek package insert. There are no contra-indications for Lymphoseek. In clinical trials and subsequent to FDA approval in March 2013, there have been no reported hypersensitivity reactions or serious adverse events.

5.2.2.1. Adverse Reactions

None of the 553 subjects studied with either breast cancer, melanoma or SCC of the oral cavity, lip or skin that received Lymphoseek experienced serious adverse reactions. Injection site irritations [0.7% (4 subjects)] and pain [0.2% (1 subject)] were reported (28–30,32–34).

5.2.2.2. Precautions

Hypersensitivity reactions: Lymphoseek might pose a risk of hypersensitivity reactions due to its chemical similarity to dextran. Serious hypersensitivity reactions have been associated with dextran and modified forms of dextran (such as iron dextran drugs).

Prior to administration of Lymphoseek, subjects should be asked about previous hypersensitivity reactions to drugs, in particular dextran and modified forms thereof. Resuscitation equipment and trained personnel should be available at the time of Lymphoseek administration, and subjects observed for signs or symptoms of hypersensitivity following injection.

5.3 Availability

Panitumumab-IRDye800 is being manufactured at the GMP biologics facility at the Frederick National Laboratory by the NCI NExT Program using cGMP IRDye800CW and commercially-available panitumumab. Formal testing of the panitumumab-IRDye800 for stability, sterility and antigen specificity is contained within the IND CMC section according to a FDA-approved process. The manufacturing and quality processes are documented elsewhere.

Lymphoseek kits are commercially available through Navidea Biopharmaceutical, Inc.

5.4 Agent Ordering

Frederick National Laboratory by the NCI NExT Program will ship panitumumab-IRDye800 as an investigational agent, to be stored at the investigational pharmacy under a material transfer agreement. The investigational pharmacy will be responsible for storage and dispensing of the

drug following a standard institutional process after the subject is screened and enrolled in the study.

Lymphoseek is supplied as a unit dose from Cardinal Health.

5.5 Agent Accountability

The Protocol Director (PD) will be responsible for the accounting of panitumumab-IRDye800. The documentation of lot numbers, dose and administration records and storage of drug will be the responsibility of the PD as well, and are described in detail in the CMC documentation. The PD will be responsible for ensuring study drug is correctly labeled and the appropriate dose provided per the protocol.

6. DOSE MODIFICATIONS

There are no dose modifications allowed in this study.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Adverse Event (AE)

An adverse event (AE) 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 SAE is defined (21CFR312.32) 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 PD up to 30 days after the study infusion and treated as a SAE with regard to the reporting time frame to Sponsor. Any pregnancy must be followed until 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, data regarding the outcome of this pregnancy will be collected after obtaining informed consent of the pregnant individual.

7.3 Assessment of Causality

For any AE 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.

7.4 Severity of Adverse Events

AEs will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0) (http://evs.nci.nih.gov/ftp1/CTCAE/about.html).

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

7.5 Safety Monitoring and Reporting

7.5.1. Monitoring and Recording of Adverse Events

Both serious and non-serious AEs will be clearly noted in source documentation and listed on study-specific Case Report Forms (CRFs). The PD or designee will assess each AE to determine whether it is unexpected according to the Informed Consent, Protocol Document, or 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 occur within 30 (+/-7) days after surgical resection of the tumor; 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 Day 1 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 v.5.0 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 days after the last dose of the study treatment.

7.5.2. Reporting Adverse Events and Serious Adverse Events

Non-serious adverse events will be reported annually to FDA via an Annual Report (IND 119474) and to IRB via Continuing Review.

The PD (aka Principle Investigator) must immediately report within 24 hours of knowledge of event to the Sponsor (ie, IND Holder, Eben Rosenthal, MD), any serious adverse event, whether or not considered related to study drug. SAEs of all grades will be reported to the Sponsor 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 an "Unanticipated Problem" (UP) 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 (ie, not described in the protocol, Investigator's Brochure or informed consent documents) and related to study drug must be reported to Sponsor using the FDA MedWatch form 3500a within 24 hours of knowledge of event. The Sponsor is responsible for deciding whether the event meets IND Safety Reporting criteria.

Events will be submitted to Sponsor Dr. Eben Rosenthal, at:

Eben Rosenthal, M.D
Professor of Otolaryngology-Head and Neck Surgery & Radiology
875 Campus Dr
Room: CC-2213, M/C 5739
Stanford, CA 94305
650-723-2967

It will be the responsibility of the Sponsor 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 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

Confirm our phase 1 study in HNSCC (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 (including, but not limited to, photoacoustic imaging); 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 Table

Enrolled subjects will follow schedule of evaluations provided below:

	Screening/ Pre-treatment (≤ 30 days before treatment)	Day 0	Day 0 to 4	Day of Surgery (Day 1 to 5)	Day of discharge[12]	Day 15 (±4 days[12])	Day 30 (±4 days)
Informed Consent	X						
Medical History	X						
Physical Examination [1]	X	X			X	X	
Vital Signs	X	X	X	X	X	X	
Height and weight	X	X					
Performance Status [1]	X				X	X	
ECG	X [2]						
Chemistries [3]	X	X		X		X	
Hematology [4]	X					X	
Serum pregnancy [11]	X	X [11]					
PK - fluorescent-labeled antibody [5]		X	X [5]	X	X	X	
PK – Immunogenicity [6]		X				X	
IV infusion of Panitumumab-IRdye800 [7]	X						
Local administration of Lymphoseek [13]			X				
Sentinel node mapping (lymphoscintigraphy + SPECT/CT imaging) [13]			X				
Surgical Resection				X [8]			
Tumor imaging				X [9]			
Pathological evaluations [10]				X			
Adverse events [15]		X	X	X		X	X
Concurrent medications [14]	X	X	X	X		X	X

1. A physical examination and performance status assessment will be done at screening; Day 0; Day of discharge; and Day 15. Examinations collected for surgical procedure are acceptable for screening purposes. Examinations do not have to be repeated on Day 0 if completed within 21 days of infusion.
2. 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 screening ECG as long as it was obtained within 21 days of infusion.
3. 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 of infusion. On Day of Surgery and Day 15 only magnesium and phosphorus are collected.
4. Hematology (ie, CBC W/O DIFF) values include WBC; RBC; Hgb; hematocrit and platelet counts. Blood work collected for surgical procedure are acceptable for screening purposes.

5. Serum level of panitumumab-IRDye800 will be drawn on Day 0 prior to dose and 30 (± 10) minutes after the end of infusion, on Day 1 if applicable, day of surgery, up to once a day on Days 1, 2, and 3 post-operative as available, Day of discharge, and Day 15 to assess circulating fluorescent-labeled antibody and unconjugated dye.
6. Blood will be obtained pre-infusion and 15 days after infusion and banked for future immunogenicity testing. Immunogenicity testing time points will not vary with dose.
7. Infusion of study drug to be given over 15 (± 5) minutes followed by a 30 (± 10) minute observation period.
8. Surgical excision occurs 1 to 5 days after the panitumumab-IRDye800 infusion based on subject availability/scheduling.
9. Tumors will be imaged intraoperatively using the devices listed in Appendix B to determine threshold of detection.
10. All resected tissues will be evaluated for near-infrared fluorescence (NIR) fluorescence prior to and after pathological examination.
11. Serum pregnancy test will be obtained on all women of childbearing age at screening; 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.
12. Blood will also be obtained as needed to evaluate any abnormal lab values experienced after study drug infusion. Blood will not be taken at Day of Discharge if it is within the window of their Day 15 visit.
13. Lymphoseek will be administered locally on the day before surgery. Directly after injection, lymphoscintigraphy will be performed to identify the drainage pattern of the tracer and to identify hot spots (ie, sentinel nodes). SPECT/CT imaging will be performed to determine the anatomical location of the hot spots.
14. Concurrent medications will be reported at screening, Day 0 and Day 15, and in-between if any medication is given to treat adverse events or serious adverse events that in the assessment of the PI are possibly, probably or definitely related to a study procedure.
15. See Section 7.5.1 for adverse events monitoring and reporting.

9.2 Study Procedures

The following evaluations and procedures will be performed:

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

- Obtain written informed consent
- Medical history including current medications
- Physical examination
- Performance Status
- ECG
- Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Height and weight
- Chemistries (standard basic metabolic profile with magnesium and phosphorus)
- CBC and platelets
- Serum pregnancy test for women of childbearing potential
- Assessment for concomitant medications
- Collect data from any standard-of-care information, including, but not limited to MRI, and PET/CT, performed prior to surgery

9.2.2 Day 0

- **Prior to Infusion of panitumumab-IRDye800**
    - Physical Examination (within 21 days)
    - Performance Status (within21 days)

- Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Chemistries (standard basic metabolic profile with magnesium and phosphorus, within 21 days)

  (may skip if previous assessment within 14 days)
- Pregnancy test for women of childbearing potential - 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
- Blood sample for panitumumab-IRDye800 antibody pharmacokinetics
- Blood sample for future assessment of immunogenicity

- **IV infusion of panitumumab-IRDye800 over 15 (± 5) minutes**
  - Vital signs immediately following panitumumab-IRDye800 infusion (+ 5 mins) (blood pressure; heart rate; respiratory rate; temperature)
- **Observation for 30 (± 10) minutes**
- **At the end of the observation period (ie, 30 (± 10) minutes after the end of panitumumab-IRDye800 infusion)**
  - Vital signs (blood pressure, heart rate, respiratory rate, temperature)
  - Blood sample for panitumamab-IRDye800 antibody pharmacokinetics
  - Assessment for concomitant medications
  - Assessment for adverse events

9.2.3 Day 0 to -4 (Day before Surgery) – for Cohort 1 only

- Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Blood sample for panitumumab-IRDye800 antibody pharmacokinetics
- Local Lymphoseek injection
- Preoperative LN mapping using lymphoscintigraphy and SPECT/CT imaging
- Assessment for adverse events
- Assessment for concomitant medications

9.2.4 Day 1 to 5 (Day of Surgery)

- Vital signs prior to surgery (blood pressure, heart rate, respiratory rate, temperature)
- Serum magnesium and phosphorus
- Blood sample for panitumumab-IRDye800 antibody pharmacokinetics
- Scheduled routine surgical resection based on standard-of-care histopathology
- Intraoperative imaging (fluorescence signal detection)
- Intraoperative gamma tracing (radioactive signal detection) (Cohort 1 only)

- 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 medication

9.2.5 Day of Discharge

- Physical examination
- Vital signs
- Performance Status
- Blood sample for panitumumab-IRDye800 antibody pharmacokinetics
- Assessment for adverse events
- Assessment for concomitant medications

9.2.6 Day 15 (± 4 days)

- Physical examination
- Vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Performance Status
- Serum magnesium and phosphorus; any other blood tests as needed to assess for any ongoing abnormalities, per PIs discretion
- CBC and platelets
- Blood sample for future assessment of immunogenicity
- Blood sample for panitumumab-IRDye800 antibody pharmacokinetics
- Assessment for adverse events
- Assessment for concomitant medications

9.2.7 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 CBC; platelets; basic metabolic profile; magnesium and phosphorus; and serum pregnancy if necessary. Blood will be taken for fluorescent-labeled antibody pharmacokinetics and 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. 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; 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 Cardiac Monitoring: An ECG will be obtained during the screening period. 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. QT/QTc interval will be assessed at screening.

10. MEASUREMENTS

10.1 Primary Outcome Measure

The **primary objective** of the study is to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck LNs of subjects with HNSCC.

The **primary endpoint** is: The number and location of panitumumab-IRDye800 positive LNs that are also tumor-positive at (histo-)pathology.

10.2 Secondary Outcome Measure

The **secondary objective** of the study is to determine if panitumumab-IRDye800 can identify LNs with the same accuracy as Lymphoseek.

The **secondary endpoint** is: The number and location of Lymphoseek positive LNs that are also tumor-positive at (histo-)pathology. Comparison of the number and location of Lymphoseek positive LNs that are also tumor-positive at (histo-)pathology vs the number and location of panitumumab-IRDye800 positive LNs that are also tumor-positive at (histo-)pathology.

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (eg, 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 PD will disseminate the protocol amendment information to all participating investigators.

The protocol and any significant amendments will be submitted to FDA. The PD is responsible for informing the Sponsor of protocol amendments.

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 PD, 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

12.1 Statistical Design

12.1.1. Primary Objective and Endpoint

The **primary objective** of the study is to determine if near-infrared fluorescence imaging of panitumumab-IRDye800 can identify metastatic disease in regional neck LNs of subjects with HNSCC.

The **primary endpoint** is: The number and location of panitumumab-IRDye800 positive LNs that are also tumor-positive at (histo-)pathology.

To **answer the primary objective**, we will calculate the sensitivity and specificity of the intraoperative findings on whether the LN is fluorescent (ie, presence of panitumumab-IRDye800) to the histopathological (gold standard) status of the LNs.

We will use the matched pair Durkalski's test (80), accounting for the cluster-correlation within subject, for all sensitivity and specificity comparisons (primary and secondary) using the clust.bin.pair package (81) in the R statistical software (84). Significance for both primary and secondary analyses will be assessed at the 0.05 level and to assess uncertainty, 95% confidence intervals will be provided for all estimates.

Descriptive statistics for continuous characteristics (eg, size of the tumor compartment) will include mean, standard deviation, median, interquartile range, minimum, and maximum values. Frequencies and percentages will be used to summarize categorical data. Graphical tools such as heat maps, histograms, and boxplots will be used to assess distributional properties of continuous variables.

12.1.2. Secondary Objective and Endpoint

The **secondary objective** of the study is to determine if panitumumab-IRDye800 can identify LNs with the same efficiency as Lymphoseek.

The **secondary endpoint** is: The number and location of Lymphoseek positive LNs that are also tumor-positive at (histo-)pathology.

To **answer the secondary objective:** We will determine if the LN tracer Lymphoseek can identify metastatic disease in regional neck LNs of subjects with HNSCC. Similar to the primary analysis, we will calculate the specificity and sensitivity of Lymphoseek for metastatic LN identification, using histology as the gold standard, and compare these to neck dissection with Durkalski's test. Since false negatives are of particular concern, we will calculate the odds of obtaining a false negative using panitumumab-IRDye800 relative to the odds of obtaining a false negative using Lymphoseek-based SNB.

12.1.3. Sample Size Justification and Power Considerations

We expect to enroll 10 T1/T2 node-negative subjects and 10 subjects with T1-4 and LN metastasis. Based on the data on LN collection from our current IRB-35064 study, we expect that 50% of subjects will undergo a bilateral neck dissection, meaning we would have a total of 30 necks. Assuming an average of 20 LN harvested per neck we would have a total of 600 LNs. Based on previous findings from our IRB-35064 study, we expect approximately 30-40% of subjects (6 to 8 of 20) will have metastatic LNs with an overall prevalence of 5 to 10% of the total number of harvested LNs (30 to 60 of 600) being tumor-positive.

Since this is a Phase 2 study, power considerations are limited but even so, we have excellent power to detect differences in specificity. 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 524 LNs. With and effective sample size of 524, a prevalence of 7.5%, a significance level of 0.05, and a 0.25 proportion of discordant pairs, we have greater than 99% power to detect a 0.1 difference in specificities, assuming the lower specificity is 0.8. Similarly, we have greater than 78% power to detect a 0.2 difference in sensitivity with the one-sided McNemar test. This assumes the proportion of discordant pairs is 0.25 and the lower sensitivity is 0.75.

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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:	**A Phase 2 Study Evaluating Panitumumab-IRDye800 vs. Sentinel Node Biopsy and (Selective) Neck Dissection for Metastatic Lymph Node Identification in Patients with Head and Neck Cancer**
Protocol Number:	IRB-43013
Principal Investigator:	Fred Baik, MD

I. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

II. 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 with recurrent disease or new primary will be allowed	☐	☐	
3. Planned standard of care surgery with curative intent for squamous cell carcinoma	☐	☐	
4. *SNB only cohort: Subjects diagnosed with a T1-T2 stage tumor, any subsite within the head and neck that is amenable to local sentinel node tracer injection and are scheduled to undergo surgical resection of the tumor, including a sentinel node biopsy	☐	☐	
5. *Neck dissection only cohort: Subjects diagnosed with any T stage, any subsite within the head and neck that are scheduled to undergo surgical resection, including a (modified) neck dissection	☐	☐	

6. Age ≥ 19 years	☐	☐	
7. Karnofsky performance status of at least 70% or ECOG/Zubrod level 1	☐	☐	
8. Have acceptable hematological status, coagulation status, kidney function, and liver function including the following clinical results: i. Hemoglobin ≥ 9 gm/dL ii. White blood cell count > 3000/mm^3 iii. Platelet count ≥ 100,000/mm^3 iv. Serum creatinine ≤ 1.5 times upper reference range	☐	☐	

*ONLY ONE CRITERION TO BE CHECKED FOR EACH PARTICIPANT - EITHER 4 or 5.

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. History of infusion reactions to monoclonal antibody therapies	☐	☐	
3. Pregnant or breastfeeding	☐	☐	
4. Magnesium or potassium lower than the normal institutional values	☐	☐	
5. Subjects receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents	☐	☐	
6. Subjects with a history or evidence of interstitial pneumonitis or pulmonary fibrosis	☐	☐	
7. Hypersensitivity to dextran and/or modified form thereof	☐	☐	

**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 – Imaging Devices Appendix

Device name	Manufacturer	Commercially available?	510(k)	Non-significant risk?	Serial No. of submission to [redacted]
Intraoperative near-infrared fluorescence imaging, in vivo					
Spy-Phi and/or PINPOINT– IR9000 (Handheld/Endoscopic)	Novadaq Inc.	Yes (modified light source for our study)	No	Yes	SN0008
Explorer Air	SurgVision	Yes (modified light source for our study)	No	Yes	SN0008
PDE-NEO II and/or FIGS	Hamamatsu Photonics K.K.	Yes	Yes	Yes	SN0012
IMAGE1 + ICG Hopkins telescope and/or VITOM	Karl Storz Endoscopes	Yes	Yes	N.A.	N.A.
Vevo LAZR-X	VisualSonics	Yes (for preclinical imaging)	No	Yes	In progress
Firefly for da Vinci	Intuitive Surgical Inc.	Yes	Yes	N.A.	N.A.
Intraoperative near-infrared fluorescence imaging, ex vivo (back table)					
Spy-Phi and/or PINPOINT– IR9000 (Handheld/Endoscopic)	Novadaq Inc.	Yes (modified light source for our study)	No	Yes	SN0008
Explorer Air	SurgVision	Yes (modified light source for our study)	No	Yes	SN0008
PDE-NEO II and/or FIGS	Hamamatsu Photonics K.K.	Yes	Yes	Yes	SN0012
IGP ELVIS v4	LI-COR Biosciences	No	No	Yes	SN0012
IMAGE1 + ICG Hopkins telescope and/or VITOM	Karl Storz Endoscopes	Yes	Yes	N.A.	N.A.
Vevo LAZR-X	VisualSonics	Yes (for preclinical imaging)	No	Yes	In progress
Firefly for da Vinci	Intuitive Surgical Inc.	Yes	Yes	N.A.	N.A.

Device name	Manufacturer	Commercially available?	510(k)	Non-significant risk?	Serial No. of submission to [redacted]
Laboratory near-infrared fluorescence imaging, ex vivo					
Pearl Triology	LI-COR Biosciences	Yes	N/A		
Odyssey CLx	LI-COR Biosciences	Yes			
IGP ELVIS v4	LI-COR Biosciences	No			
Leica fluorescence microscope	Leica	Yes			
Vevo LAZR-X	VisualSonics	Yes			
Dosimeter	Pogue Laboratory	No			
Stand-alone Firefly for da Vinci	Intuitive Surgical Inc.	Yes, on the robot			