Intraoperative Pancreatic Cancer Detection Using Multimodality Molecular Imaging

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Document History	
Version Date	**Notes**
9 February 2016	Submitted to IRB and IND
5 April 2016	• Added ECG assessment 30 minutes after the loading dose • Added the option of performing urine pregnancy test on the day of infusion if the serum pregnancy test is not within 72 hours
11 April 2016	*Ex vivo* tumor imaging process clarified
20 July 2016	Describes the use of the IR9000 cameras and the SurgVision Explorer Air. Technical information for the SurgVision Explorer Air is provided in Appendix C.
8 November 2016	• Clarifies that subjects who experience a serious reaction will be discontinued (withdrawn), and will not count towards the study accrual target specified at 12.1 Statistical Design / Sample Size • Removes the optional requirement for Day 1 follow up • Specifies that certain AEs unrelated to the study drug may not be collected, as follows. o AEs that are solely laboratory values; are not related to the study drug ; AND are not clinically significant may not be collected. o Non-serious AEs that occur within 10 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.

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PROTOCOL SYNOPSIS

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SAMPLE SIZE AND STUDY DURATION	It is anticipated that 10 subjects will be enrolled into the study (5 in each cohort). Each subject will be followed for 30 days following the last dose of study medication. Based on the enrollment rate for the current head and neck cancer Cetuximab-IRDye800 trial, it is anticipated that the study will require 12 to 18 months to be completed.
STATISTICAL CONSIDERATIONS	The primary objective is to correlate fluorescence with histological evidence of tumor relative to background tissue. The gross and histological properties of the primary tumor will be correlated with the fluorescence intensity as measured in the operating room using the intraoperative optical imaging devices Novadaq SPY/LUNA; Novadaq IR9000 Fluorescence Imaging System with Open-Field Handheld Fluorescence Imaging Camera and/or Pinpoint Endoscopic Fluorescence Imaging Camera; and SurgVision Explorer Air multi-spectral fluorescence reflectance system. A board certified pathologist will perform pathological confirmation of tumor, which will serve as the gold standard. In pathology, the tumor will processed using standard of care for the purposes of patient care, these tissues or any waste tissues from the subject will be made available for optical imaging using several optical and photoacoustic imaging devices. The linear relationship between fluorescence and tumor weight will be computed as Spearman's correlation coefficient. The bias present between the measurements will be expressed in relationship to the tumor volume and fluorescence expressed as the mean difference with a 95% confidence interval. Continuous variable means (eg, fluorescence intensity) will be compared to histological quantification of tumor as previously noted and previously performed by us (Kulbersh, *et al*, 2007; Rosenthal, Kulbersh, King, Chaudhuri, Zinn, 2007; Rosenthal, *et al*, 2015). The sensitivity and specificity of these experiments will be determined based on histological confirmation of tumor within the margin specimens, as performed previously by us in preclinical models (Kulbersh, *et al*, 2007; Withrow, *et al*, 2007; Withrow, *et al*, 2008). Animal models demonstrate a sensitivity of 92% and specificity of 100% using fluorescently labeled Cetuximab (Kulbersh, *et al*, 2007). Frozen sections obtained from subjects per the standard of care will be analyzed for fluorescence intensity and the presence of tumor will be confirmed by histology. The secondary objective will be measured as the occurrence of a Dose-limiting Toxicity (DLT) event, defined as any Grade 2 or greater toxicity that is considered possibly or definitely related to the study drug, is considered clinically significant by the investigator and occurs within 15 days of receiving treatment.

	Subjects will be monitored using standard clinical parameters to evaluate for toxicity. Subjects will be observed immediately after infusion of the unlabeled test/loading dose for an infusion reaction to Cetuximab. If there is no serious reaction, the subject will be enrolled in the study and receive the study drug. Subjects who experience a serious reaction will be discontinued (withdrawn), and will not count towards the study accrual target specified at 12.1 Statistical Design / Sample Size. The following observations and tests shall be performed after treatment to identify both acute and late treatment specific toxicity: physical exam, toxicity grading, assignment of performance status, and at certain intervals blood chemistries (complete metabolic profile to include magnesium and phosphorus). NCI Common Toxicity Criteria v 4.0 will be used for grading of toxicity. Safety endpoints are type, incidence, severity, seriousness, and study treatment relatedness. Descriptive statistical analysis of subject disposition, baseline characteristics, exposure to study drug, and adverse events (AEs) will be performed. Descriptive statistics for continuous data will include mean, standard deviation, median, minimum, and maximum values. Frequencies and percentages will be used to summarize categorical data.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
BID	Twice Daily
BSA	Body Surface Area
CBC	Complete Blood Count
CI	Confidence Interval
CMAX	Maximum Concentration Of Drug
CNS	Central Nervous System
CRF	Case Report/Record Form
CTCAE	Common Terminology Criteria For Adverse Events
DLT	Dose-Limiting Toxicity
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
ECOG	The Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
FDA	Food and Drug Administration
GI	Gastrointestinal
GMP	Good Manufacturing Practice
Hgb	Hemoglobin
HNSCC	Head And Neck Squamous Cell Carcinoma
HTN	Hypertensions
ICG	Indocyanine green
ILD	Interstitial Lung Disease
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower Limit Of Normal
MTD	Maximum Tolerated Dose
NIR	Near Infra-Red
NHS	N-hydroxysuccinimide
PAI	Photoacoustic imaging
PDAC	Pancreatic ductal adenocarcinoma
PLT	Platelet
ROI	Regions of Interest
SAE	Serious Adverse Event
SCC	Squamous Cell Carcinoma
SNR	Signal to Noise Ratio
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 Objectives

The primary objective of the study is to determine the efficacy of Cetuximab-IRDye800 in intraoperatively identifying pancreatic cancer compared to surrounding normal pancreatic and extrapancreatic tissue, as measured by tumor-to-background ratio.

1.2. Secondary Objectives

The secondary objective is to determine the tolerability of the Cetuximab-IRDye800 as an imaging agent in subjects undergoing resection of pancreatic cancer.

2. BACKGROUND

Need for optical imaging to guide surgical resection of pancreatic cancer. There is currently no pancreatic cancer specific contrast agent available for preoperative and/or intraoperative imaging. Despite significant investment in systemic therapy for small incremental gains in survival, there has been minimal investment in improving surgical outcomes despite the overwhelming number of patients who undergo surgical resection for pancreatic cancer every day in the United States.

We propose to leverage previous investment in systemic therapy to improve the completeness of surgical resection and improve the margin positivity rate, which can be as high as 85% in certain series with standardized pathological processing (Chang, *et al*, 2009; Esposito, *et al*, 2008; Kooby, *et al*, 2014; Van den Broeck, *et al*, 2009; Verbeke and Gladhaug, 2012; Wagner, *et al*, 2004; Winter, *et al*, 2006). This 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. Successful completion of this study would have a lasting impact on the field of surgical oncology.

2.1 Study Disease

Importance of Margin Clearance in Pancreatic Cancer. Pancreatic cancer is the fourth leading cause of cancer death in the Western world, with 5-year survival rate of less than 5% (Jemal, *et al*, 2010). At diagnosis, 75 to 85% of patients are not eligible for resection, due to early locoregional and/or distant spread (Barugola, *et al*, 2009). Two major predictors of long-term survival are the ability to resect the tumor with cancer-free resection margins and the absence of systemic metastases. However, margin-positive resections are a frequent phenomenon (Verbeke, Gladhaug, 2012) as is the emergence of distant metastases soon after surgery (which were likely present at the time of surgery in the form of non-detectable micrometastases). These factors create significant challenges for the proper selection of patients for surgery, as well as for decision-making during the actual procedure in order to achieve complete clearance of the tumor or abort a non-curative resection.

Pancreatic cancer can be difficult to visualize intraoperatively. Non-curative resection has been a significant limitation in the management of pancreatic cancer given the lack of intraoperative tools to discriminate between benign/inflammatory pancreatic parenchyma versus pancreatic cancer tissue. This limitation results in:

1. Incomplete resection of tumors, which could have been resected completely
2. Incomplete resection of tumors that could have been identified at operation as unresectable if the local extent of tumor growth were assessable.
3. Incomplete lymph node clearance due to lack of knowledge of involved or potentially involved lymph nodes.
4. Resection of pancreatic tumors in the presence of visually or radiographically occult systemic metastasis (liver or peritoneum).

In these situations, patients undergo operations with reduced oncologic benefit, but with a high risk of deterioration of quality of life due to a major operation at an end stage of life. The use of high-targeted molecular imaging has the potential to provide crucial information to surgeon in the above-described situations, both in the form of photoacoustic imaging, which can provide intraoperative ultrasound images at clinically relevant depths (5 to 7 cm) with high special resolution (Zackrisson, van de Ven, Gambhir, 2014), and in the form of fluorescent optical imaging, which on the other hand is superior for imaging superficial lesions (Vahrmeijer, Hutteman, van der Vorst, van de Velde, Frangioni, 2013).

Intraoperative frozen sections are used to confirm complete resection of tumors. Frozen section analysis of specimen margins has significant deficiencies, as it 1) requires resection of additional tissue for assessment; 2) adds between 30 and 60 minutes to the operative time; 3) is difficult to interpret in patients with other pancreatic diseases or previous irradiation; 4) cannot be performed on fat; 5) is reversed on permanent section in approximately 5% of cases and 6) fails to detect close margins (< 5 mm) (DiNardo, Lin, Karageorge, Powers, 2000). Finally and perhaps most importantly, ***frozen sections are highly accurate if the right tissue is biopsied and sent, however, less than 5% of the resection bed can be assessed by frozen section and this significantly limits the sensitivity of this technique.*** This sampling error consistently plagues surgeons and pathologists alike.

Current intraoperative hardware can successfully image the Cetuximab-IRDye800 in vivo. Novadaq currently manufactures an FDA-approved intraoperative device for imaging intravascular flow of indocyanine green in plastic surgery procedures. These systems include the SPY or LUNA system, the PINPOINT device for laparoscopic imaging, and the FIREFLY for robotic assisted imaging. Although not specifically designed to image Cetuximab-IRDye800, the emission and absorption spectrum of these two optical agents (indocyanine green and IRDye800) have significant overlap and we present data to show that this system can successfully detect microscopic disease in preclinical models (Heath, Deep, Sweeny, Zinn, Rosenthal). Development and manufacturing of new hardware for the purpose of specifically imaging Cetuximab-IRDye800 is currently done by Novadaq, creating the IR9000 Fluorescence Imaging System. However, no clinical study is yet done with that device. Similar technologies are being incorporated into minimally invasive technologies, which are ideally suited to optical imaging.

Will tissue penetration be sufficient to identify cancer? Indocyanine green is a fluorescent dye with very similar properties to IRDye800 that is routinely used in humans to evaluate

vascular flow in the surgical setting with strong penetration through skin and other structures (Yamamoto, *et al*.). Furthermore, indocyanine green has been successfully administered at microdosing levels to study lymphatic drainage in humans (Rasmussen, Tan, Marshall, Fife, Sevick-Muraca, 2009; Sharma, *et al*, 2008). Unfortunately, indocyanine green does not have a function group for conjugation to other proteins. However, IRDye800 is a stable near infrared imaging dye that can be conjugated to antibodies without significant alteration of ligand binding. We chose this dye because it is the only optical dye we are aware of that has undergone safety testing and is being evaluated by the FDA for use in humans.

Improved visualization would significantly improve minimally invasive surgical techniques. Optical imaging is ideally suited to endoscopic and laparoscopic/robotic operative approaches since the surgeon is looking directly at the video monitor and fluorescence can be easily incorporated into the surgeons view. Furthermore, most current versions of intraoperative microscopy recognize the importance of future applications of fluorescence and have developed systems to image at a wavelength that could easily accommodate Cetuximab-IRDye800, such as the OH5 FL800 (Leica Microsystems) and Pentero 900 (Carl Zeiss) microscopes.

EGFR is highly expressed in pancreatic ductal adenocarcinoma (PDAC) and is a good target for fluorescence imaging, due to its transmembrane position. Cancer-specific surgical navigation has been successfully introduced for certain cancer types and it requires accurate localization of vector molecules to cancer cells. In the past, studies demonstrated image-guided pancreatic surgery using, the non-tumor specific near-infrared dye, indocyanine green. They showed that no useful tumor demarcation could be visualized in pancreatic cancer patients, most likely due to the non-specific character of the dye (Hutteman, *et al*, 2011). This clearly delineates the importance of using a tumor-specific imaging agent for pancreatic cancer, to improve patient outcomes by enhancing accurate staging, and more effective, tumor-margin free pancreatic resection. However, to date no clinical studies with tumor-specific imaging agents for pancreatic cancer have been performed.

Studies have shown EGFR overexpression in pancreatic cancer to range from 30-95% (Bloomston, Bhardwaj, Ellison, Frankel, 2006; Uegaki, *et al*, 1997). And certain EGFR-directed therapies are currently FDA approved for pancreatic cancer treatment, such as erlotinib (Kelley, Ko, 2008; Philip, Lutz, 2015). Preliminary studies from our research group showed that epidermal growth factor receptor (EGFR) is a promising target for pancreatic cancer. In a cohort of 129 patients with different stages of PDAC, EGFR was abundantly expressed in up to 70% of pancreatic cancer samples (*de Geus, et al. Manuscript in preparation*). To validate these results, we also tested the markers on tissues of patients with chronic pancreatitis and in lymph node metastases. These experiments confirmed that EGFR is an appropriate tumor-specific marker, also able to differentiate between benign and malignant pancreatic lesions, which is crucial in pancreatic surgery (*Tummers, et al. Manuscript in preparation*).

In addition, research efforts continue to elucidate a possible role for cetuximab in the treatment of pancreatic cancer. Cetuximab has shown anti-tumor activity either alone or in combination with other agents and is currently FDA approved for use in both head and neck squamous cell carcinoma (HNSCC) and colorectal carcinoma. Other studies have established the safety profile of Cetuximab in pancreatic cancer patients (Arnoletti, *et al*, 2011; Ho, *et al*, 2011).

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 the fluorescently labeled anti-EGFR antibody (Day, Beck, Heath, *et al*, 2013; Day, Sweeny, Kulbersh, Zinn, Rosenthal, 2013). 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 (Agnes, *et al*, 2012; Kobayashi, *et al*, 2012; Qi, *et al*, 2012; Yang, *et al*, 2013).

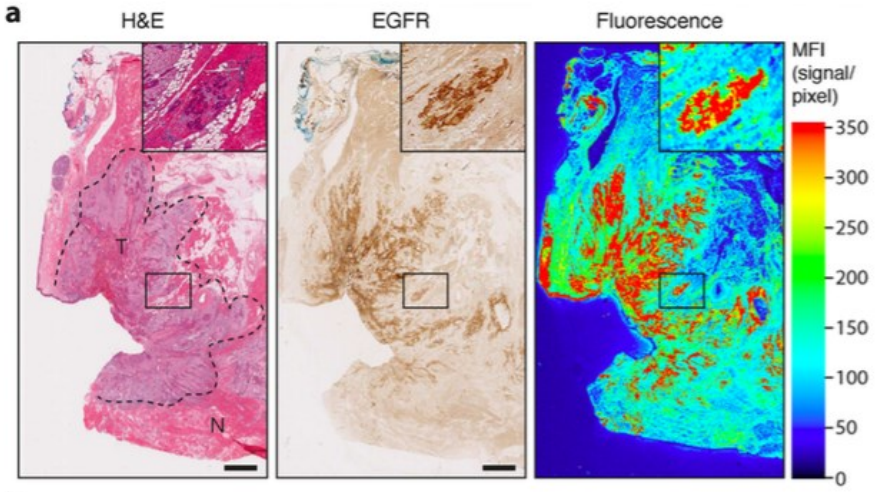


Figure 1. Co-localization of fluorescence signals of Cetuximab-IRDye800CW and Epidermal Growth Factor Receptor (EGFR) expression.

(**a**) Representative haemotoxylin/eosin (H&E) image indicating tumor (T) and normal (N) with corresponding EGFR expression immunohistochemistry stain and fluorescence image.

(**b**) Increase in Mean Fluorescence Intensity (MFI) as a function of EGFR Density (% area EGFR positive). Data are presented as mean±SD, * = P<0,001. Scale bars in all images represent 100µm.

Do tumors need to express high levels of EGFR for successful fluorescence imaging? Early data from clinical trials evaluating the relationship between fluorescence intensity and EGFR has suggested that 1) Mean Fluorescence Intensity (MFI) correlates with the expression of EGFR and 2) even very low levels of EGFR expression are sufficient for strong fluorescent signal (Figure 1). Analysis of human tissues after systemic injection of Cetuximab-IRDye800 in head and neck cancer patients revealed that EGFR expression correlated with mean fluorescence intensity (MFI). Clearly, Cetuximab-IRDye800CW uptake within SCC was specifically localized to areas that expressed EGFR. The univariate association between EGFR density and MFI was observed at all three dosing cohorts ($P < 0.001$, Figure 1b). A 6-fold increase in MFI was seen at the two higher doses compared to the lower dose concentration (P<0.001); no

significant increase in MFI was seen when comparing the highest dose (62.5 mg/m^2) with the middle dose 25 mg/m^2. ($P > 0.05$).

2.2 Study Agent/Device/Procedure

Cetuximab-IRDye800 is produced by mixing the two components in phosphate buffer 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. Cetuximab-IRDye800 has been produced under cGMP conditions at University of Alabama at Birmingham using cGMP IRDye800CW and commercially available cetuximab. Formal testing of the Cetuximab-IRDye800 for stability, sterility and antigen specificity is contained within the CMC section according to an FDA-approved process. The manufacturing and quality processes are documented elsewhere.

Cetuximab-IRDye800 has recently been used for the first time in humans, in head and neck cancer patients. Comparing cetuximab with Cetuximab-IRDye800 identified very limited differences between the 2 agents, however there are several relevant findings:

1) The study demonstrated that the pharmacokinetics of single dosing with Cetuximab-IRDye800 are very similar to cetuximab; both have half-life of 2.6 days when dosed at 250 mg/m^2. Therefore data will be collected using the known pharmacokinetics of cetuximab.
2) A small but statistically significant increase in the QTc interval was identified during the infusion of cetuximab or Cetuximab-IRDye800. Therefore patients at risk for arrhythmias associated with QT prolongation will be excluded (see exclusion criteria).

For clinicaltrials.gov compliance

The investigational agent for this study, Cetuximab-IRDye800, is not approved by the 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 Erbitux (cetuximab).

The IND for this study is IND-115706.

2.3 Rationale

Overall Impact of Proposed Studies on Surgical Oncology. Introduction of this technology to the operating room in the manner we propose would represent a paradigm shift in oncologic surgery. Current methods for detecting surgical margins are subjective (surgeon's inspection and palpation) and time consuming (frozen section analysis). The use of optical imaging would be a significant improvement in real-time identification of cancer – when the surgeon can act on the information and extend the resection to achieve negative margins. The development of a cancer specific contrast agent of this type can be applied to other solid tumors that express EGFR. Furthermore, if the principle of targeted optical imaging is validated using EGFR, other therapeutic antibodies could be assessed for imaging potential.

Proposed studies will dramatically improve current standard of care in following ways:

1) Detect subclinical disease in the operating room at the time of resection: Our preclinical data suggests tumor volumes of greater than 450 cells are detectable by this technique (Rosenthal, Kulbersh *et al*. 2006, Rosenthal, Kulbersh *et al*. 2007).

2) Real time feedback: Tumor margins and then residual disease can be assessed intraoperatively.

3) Improve minimally invasive techniques: Use of endoscopes in body cavities with limited light represents ideal conditions for this technology.

4) Use in other cancer types: EGFR is highly-expressed in multiple cancer types, allowing application to other tumor types beyond pancreatic cancer and HNSCC.

5) Potential other clinical applications: Cancer detection by laparo-endoscopic techniques during screening or post-treatment surveillance(Gleysteen, Duncan, *et al*, 2007b).

6) High feasibility. We have previously conducted toxicity studies in non-human primates (Zinn, *et al*, 2015) and in a dose escalation phase 1 clinical trial using Cetuximab-IRDye800 in head and neck cancer patients (Rosenthal, *et al*, 2015).

2.4 Study Design

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance:

- The primary purpose for the protocol is **Image Guidance**. This study evaluates if Cetuximab-IRDye800, a dye conjugate intended to identify cancer compared to surrounding normal tissue, improves intraoperative identification of pancreatic cancer vs surrounding normal pancreatic and extrapancreatic tissue, as measured by tumor-to-background ratio.
- There are **2 cohorts**.
- The study is **Open-label**. No masking is used.
- The study is **non-randomized**.
- The primary outcome is **Efficacy**.

The study is an open-label study to determine the efficacy and safety profile of conjugated Cetuximab-IRDye800 used in subjects with pancreatic cancer that undergo surgery. This study aims to evaluate the specificity of cetuximab conjugated to the fluorescent dye (IRDye800CW) in the intraoperative detection of micrometastatic disease in the peritumoral lymph nodes, primary tumor, and other distant sites.

Previous data in nonhuman primates (n = 8) and in humans (n = 15) suggest that Cetuximab-IRDye800 behaves similarly to unlabeled cetuximab, but its efficacy and safety remains largely unknown because of the limited clinical experience (Rosenthal, *et al*, 2015; Zinn, *et al*, 2015). The manufacturing places 1 or 2 dye molecules per antibody, which is theorized to have minimal impact on the pharmacodynamics and toxicity of the antibody-dye compared to the antibody alone. These studies have suggested that the risk of Cetuximab-IRDye800 at this sub-therapeutic dose is similar but not equivalent to the toxicity of cetuximab.

A total of 10 subjects will be enrolled in the proposed study. During and after Cetuximab-IRDye800 infusion, allergic reactions will be monitored and safety measurements will be taken, such as hemodynamic monitoring, EKG, blood tests, and physical examination. 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 7 days of

receiving treatment, which represents more than 4 half-lives of the drug. Restarting will require reassessment after discussion and approval by the medical monitoring board.

We will administer a test/loading dose of unlabeled cetuximab (no dilution required) over 30 minutes via IV and observe for 30 minutes. The administration of this dose of unlabeled cetuximab has two purposes: 1) a test dose will allow toxicity related to cetuximab alone to be differentiated from hypersensitivity reactions associated with Cetuximab-IRDye800, and 2) Because the liver acts as an EGFR 'sink,' a loading dose of cetuximab is given as standard of care in therapeutic delivery of the drug. Because of this, we have proposed the use of a 100 mg pre-treatment dose to saturate some of the liver receptors prior to administration of Cetuximab-IRDye800. Tumor saturation with the test dose is unlikely based on our preclinical studies of tumor cells in vitro (Helman, *et al*.) and in vivo (Gleysteen, *et al*, 2007). Our preclinical studies with excess unlabeled anti-EGFR support this conclusion as tumor retention of fluorescent-anti-EGFR antibody was still imaged under these conditions (Gleysteen, *et al*, 2007).

Subjects will be assigned in 2 cohorts. Cohort 1 will receive by IV infusion a 100 mg unlabeled test/loading dose of cetuximab and 50 mg of Cetuximab-IRye800. Cohort 2 will receive by IV infusion a 100 mg unlabeled test/loading dose of cetuximab and 100 mg of Cetuximab-IRDye800. The rationale for these doses are based on preliminary results for our phase 1 clinical trial (Rosenthal, *et al*, 2015) which demonstrated 1) 50 mg dose provides the highest tumor-to-background ratio compared to other doses; 2) 100 mg dose is within the range of head and neck cancer trial and is based on the difference between the tumor types, looking at anatomical localization, the need for detection of micrometastases, and positive lymph nodes; and 3) the 100 mg unlabeled test/loading dose is optimal in reducing normal tumor background.

Cohort	Number of Subjects	Dose Cetuximab-IRDye800	Loading Dose unlabeled cetuximab	Time between infusion and surgery
1	5	50 mg	100 mg	2 to 5 days
2	5	100 mg	100 mg	2 to 5 days

Pharmacokinetic Intervals. Because the pharmacokinetics of single dose cetuximab are well known and the pharmacokinetics of cetuximab and Cetuximab-IRDye800 were nearly the same in non-human primate studies (see toxicology section), only limited pharmacokinetics will be performed (Tan, *et al*, 2006). Blood levels will be monitored before infusion (up to 30 days before), after infusion (2 h), on Day 1 as available, on Day 2 to 10, and on Day 15 to 30 for Cetuximab-IRDye800 and free IRDye800. Clearance of cetuximab occurs through the binding in the liver and through the reticuloendothelial system as occurs with endogenous immunoglobulin.

Subjects will undergo surgical resection 2 to 5 days after infusion of Cetuximab-IRDye800. Initial estimates of timing of surgery were based on preclinical findings which show peak imaging time approximately 3-5 after injection which allows time for the antibody to accumulate within the tumor (Day, Beck, Deep, *et al*, 2013; Day, Beck, Heath, *et al*, 2013; Day, Sweeny, *et al*, 2013; Heath, Deep, Sweeny, Zinn, Rosenthal, 2012) and later confirmed by human studies (data not shown). Intraoperatively, tumor imaging will be performed in vivo to assess optimal tumor-to-background ratio (TBR). TBR is defined as fluorescent signal of tissue

compared to florescent signal of tissue surrounding the tumor at different doses. The number and location of detected lesions by conventional and optical techniques will be recorded on a schematic case-report form (CRF). After surgery, non-invasive investigational optical and photoacoustic imaging modalities will be used without violation of the tissue. With these modalities both TBR and the signal-to-noise ratio measured in dB (SNR) of the tumor will be calculated in the region of interest (ROI) and compared to normal surrounding tissue. This will be done to determine if those imaging techniques are complementary to each other and thereby an advantage for pancreatic cancer surgery. In addition, routine hematoxylin and eosin (H&E) staining will be done for histologic assessment and correlated with optical imaging. Immunohistochemistry on unstained tissue sections of the tumor and normal tissue will be performed as well as evaluate EGFR-expression. The standard of care will not be violated during the surgical management or the pathological processing of tissues.

Subjects will be monitored using standard clinical parameters to evaluate for toxicity. Subjects will be observed immediately after infusion of the unlabeled test/loading dose for an infusion reaction to cetuximab. If there is no serious reaction and the subject receives the full test dose of unlabeled cetuximab, the subject will continue on-study and receive the study drug Cetuximab-IRDye800. The following observations and tests shall be performed after treatment to identify both acute and late treatment-specific toxicity: physical exam; toxicity grading; ECG; assignment of performance status; and at certain intervals blood chemistries (complete metabolic profile to include magnesium and phosphorus). NCI Common Toxicity Criteria v4.0 will be used for grading of toxicity.

Imaging. Imaging will occur intra-operatively and during pathological processing (see Figure 2 below).

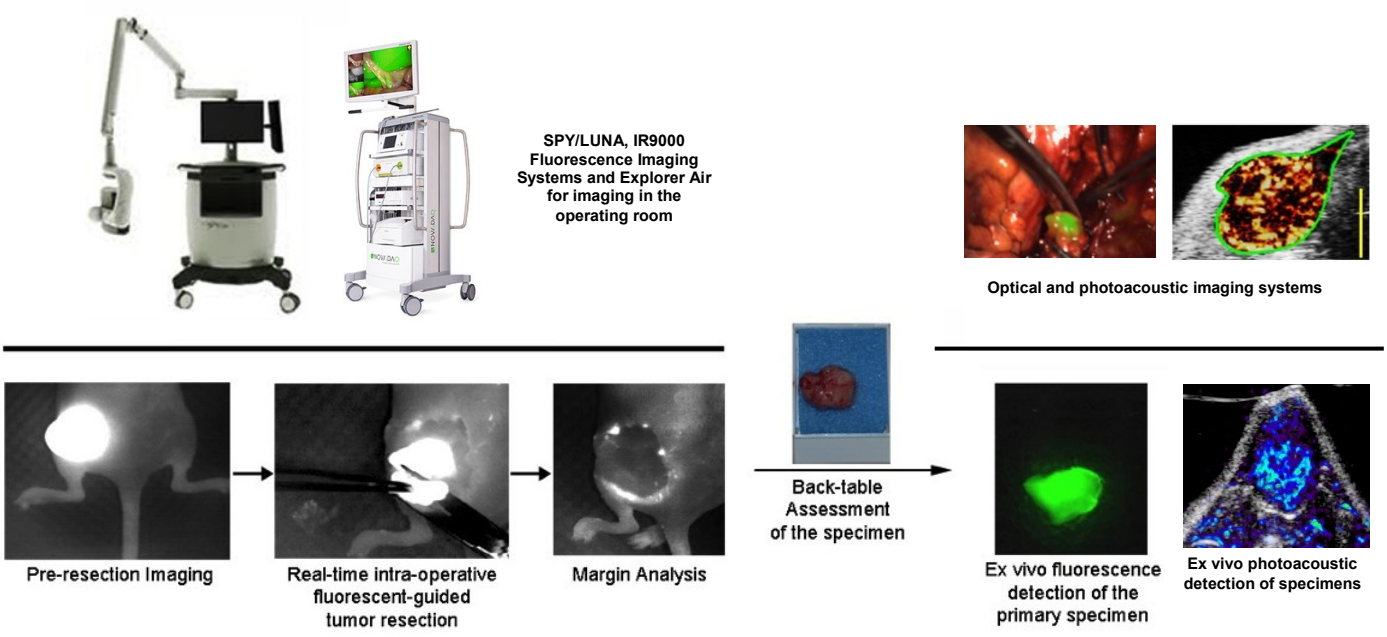


Figure 2. Schematic of intraoperative and pathological imaging of cancer to assess surgical margins. Anti-EGFR antibody conjugated to IRDye800 was injected systemically into an immunodeficient mouse bearing a xenografted squamous cell carcinoma and imaged after 48 hours.

***In Vivo Fluorescent Imaging*.** An optical imaging system (approved optical device or IRB approved device) will be used to image the tumor in the operating room. These devices are FDA approved for measuring intraoperative blood flow after indocyanine green (ICG) injection. Because IRDye800 and ICG have overlapping excitation and emission spectra, the SPY/LUNA System and PINPOINT based IR9000 Fluorescence Imaging System, Novadaq, can be used for imaging subclinical disease based on our preclinical studies in head and neck cancer (Heath, *et al*). The IR9000 Fluorescence Imaging System, Novadaq, is based on the Novadaq's commercial PINPOINT Endoscopic Fluorescence Imaging System (PC9000), see Appendix B. Intraoperative imaging is performed with the camera head approximately 6 to 12 inches from the tumor using variable integration timing. When imaging the tumor in situ we propose to calculate tumor to background ratio (TBR). The fluorescence intensity of the tumor is compared to the grossly normal tissue imaged within the field and provides an assessment of Cetuximab-IRDye800 uptake within the tumor compared to normal tissues. Multiple measurements can be obtained at the time of surgery from a still images and histograms for regions generated using ImageJ (NIH) as we have previously described (Andrades, Rosenthal, Carroll, Baranano, Peters, 2008; Day, Beck, Deep, *et al*, 2013; Day, Beck, Heath, *et al*, 2013; Day, Sweeny, *et al*, 2013; Heath, *et al*). Intraoperative imaging will be performed with optical imaging devices Novadaq SPY/LUNA; Novadaq IR9000 Fluorescence Imaging System with Open-Field Handheld Fluorescence Imaging Camera and/or Pinpoint Endoscopic Fluorescence Imaging Camera; and SurgVision Explorer Air multi-spectral fluorescence reflectance system.

Margin analysis: The sensitivity and specificity of these experiments will be determined based on histological confirmation of tumor within the margin specimens, as performed previously by us in preclinical models (Kulbersh, *et al*, 2007; Withrow, *et al*, 2007; Withrow, *et al*, 2008).

Assessment of regional lymphatics. Subjects undergoing treatment of pancreatic cancer routinely undergo removal of additional peripancreatic lymph nodes. In subjects in whom this is required, lymphadenectomy will be performed according to the standard of care with imaging before and after removal of the lymph nodes in vivo. Imaging results (categorical positive/negative) will be correlated with histopathological evidence of disease.

***Ex Vivo Fluorescent Imaging*.** Once the tumor has been removed from the subject and sent for pathology review, additional imaging will be performed with non-significant devices 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. 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..

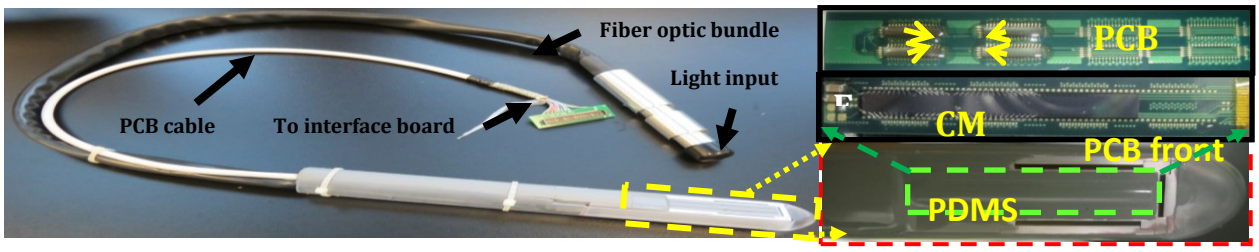
Sensitivity and specificity can be calculated from the frozen sections obtained (if removed as part of the standard of care procedure). Once removed, the dimensions and weight of the specimens will be recorded to correlate with tumor fluorescence parameters with the presence or absence of tumor based on final pathological reading. If tumor is present in the specimen quantification of tumor of the largest piece of tumor in two dimensions (mm x mm) will be performed by a board

certified pathologist. A small biopsy of the primary tumor from the main specimen will also be included in the fluorescence and pathological analysis as a positive control. Sensitivity, specificity, positive predictive value and negative predictive value will be determined as previously described by us (Rosenthal, *et al*, 2006; Rosenthal, *et al*, 2007).

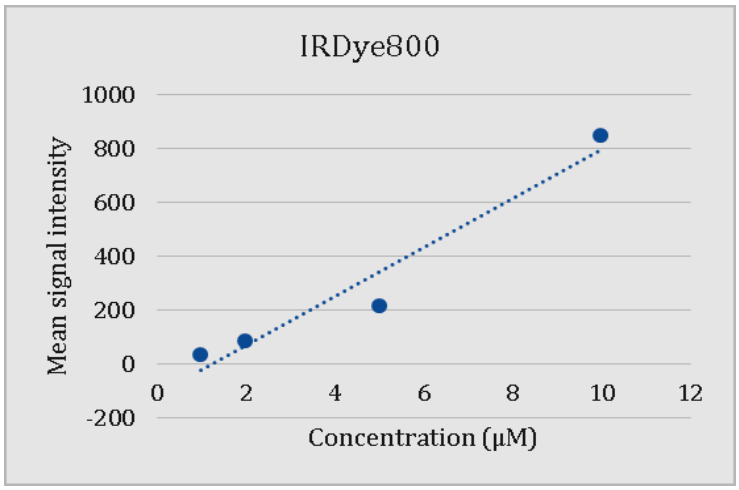
Statistical considerations: The linear relationship between fluorescence and tumor size will be computed as Spearman's correlation coefficient. The bias present between the measurements will be expressed in relationship to the tumor weight/size and fluorescence expressed as the mean difference with a 95% confidence interval. Continuous variable means (eg, fluorescence intensity) will be compared to histological quantification of tumor as measured by the greatest dimension identified on the histopathological assessment. Experiments will be performed as previously described using this method in preclinical models (Kulbersh, *et al*, 2007; Rosenthal, *et al*, 2007).

Pathological correlates: To determine biological characteristics associated with fluorescence intensity, paraffin embedded blocks from the primary tumor will be obtained from pathology after routine analysis and these histological imaged by the Pearl and Odyssey (LiCor, Lincoln, NE) as previously described in preclinical models (Heath, *et al*.). Histological sections will also undergo analysis for: 1) size of the epithelial tumor compartment in percent of total, 2) tumor viability, and 3) EGFR immunohistochemistry. Methods have been previously described by us using in vivo models (Gleysteen, *et al*, 2007b; Kulbersh, *et al*, 2007; Rosenthal, *et al*, 2006; Rosenthal, *et al*, 2007; Withrow, *et al*, 2007). These parameters will be correlated with fluorescence intensity from the Pearl and Odyssey imaging systems.

Ex Vivo Photoacoustic Imaging (PAI). The PAI device is a hand-held device (Figure 3) created at Stanford University in the Gambhir's laboratory. PAI provides optical contrast ultrasound images with high spatial and temporal resolution. PAI employs rapid short light pulses to illuminate tissue. The light is absorbed by various endogenous molecules (eg, hemoglobin) leading to minute changes in temperature, which in turn, cause the absorbing molecules to locally expand. The transient local tissue expansion generates a pressure wave (sound) that can be detected using an ultrasound imaging system. To enhance the contrast further, exogenous contrast (eg, IRDye800) can be used (Figure 3b). This instrument provides real-time, non-invasive imaging of tissues at depths of up to 5 cm at impressive clarity (Kothapalli, *et al*, 2012) (See also Appendix D). The average signal intensity (or more specifically the signal-to-noise ratio measured in dB) of the tumor will be calculated in the region of interest (ROI) and compared to normal surrounding tissue.



a.



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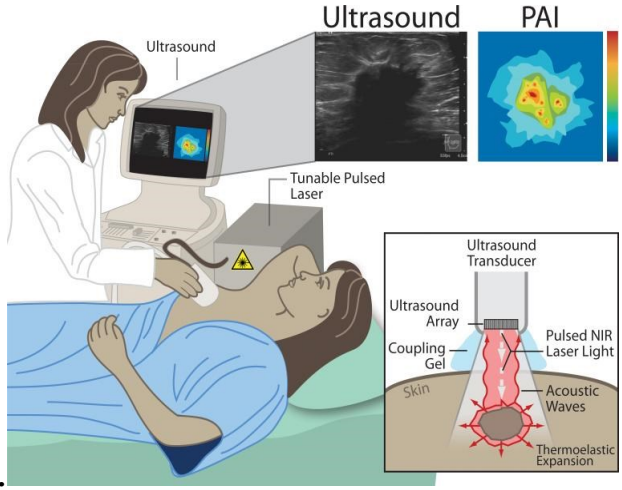

c.

Figure 3. **a**. Photoacoustic imaging (PAI) hand-held device.
b. PAI principle.
c. Phantom study of PA signal of IRDye800, showing proper PA signal even at low concentrations

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

a) Clinically suspected or biopsy-confirmed diagnosis of pancreatic adenocarcinoma.
b) Planned standard of care surgery with curative intent for pancreatic adenocarcinoma.
c) Age ≥ 19 years
d) Life expectancy of more than 12 weeks
e) Karnofsky performance status of at least 70% or ECOG/Zubrod level 1
f) Hemoglobin ≥ 9 gm/dL
g) Platelet count ≥ 100,000/mm^3
h) Magnesium, potassium and calcium > the lower limit of normal per institution normal lab values
i) TSH < 13 micro International Units/mL

3.2 Exclusion Criteria

a) Received an investigational drug within 30 days prior to first dose of Cetuximab-IRDye800
b) Myocardial infarction (MI); cerebrovascular accident (CVA); uncontrolled congestive heart failure (CHF); or unstable angina within 6 months prior to enrollment
c) History of infusion reactions to cetuximab or other monoclonal antibody therapies
d) Pregnant or breastfeeding

e) Evidence of QT prolongation on pretreatment ECG (greater than 440 ms in males or greater than 450 ms in females)
f) Lab values that in the opinion of the primary surgeon would prevent surgical resection.
g) Patients receiving Class IA (quinidine, procanamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents.

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 Registration Process

To register the subject, the study site will call the Stanford Study Coordinator at: , Monday through Friday, between 9:00 am and 5:00 pm (Pacific time). The individual making the phone call will provide the subject's eligibility information at this time. No subject may begin study treatment prior to registration, and assignment of a subject identification number.

At registration, Stanford will sequentially assign eligible subjects a unique subject identification number. As confirmation, Stanford will provide the Investigator with written verification of the subject's registration by e-mail or fax. The subject's 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 7 days.

3.5 Study Timeline

Primary Completion:

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

Study Completion:

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

The anticipated study start date: March 2016

The anticipated study completion date: March 2018

4. TREATMENT PLAN

4.1 Treatment Schedule

Patients will be randomized signed in 2 cohorts. Cohort 1 will receive a 100 mg unlabeled test/loading dose of cetuximab and 50 mg of Cetuximab-IRye800. Cohort 2 will receive a 100 mg unlabeled test/loading dose of cetuximab and 100 mg of Cetuximab-IRDye800.

Cohort	Number of Patients	Dose Cetuximab-IRDye800	Loading Dose unlabeled cetuximab	Time between infusion and surgery
1	5	50 mg	100 mg	2 to 5 days
2	5	100 mg	100 mg	2 to 5 days

4.2 Administration of Cetuximab-IRDye800

A test/loading dose of 100 mg of unlabeled cetuximab will be administered via a 30-minute IV infusion, starting about 1 hour prior to infusion of the Cetuximab-IRDye800. A fixed dose of 100 mg will be given prior to surgery. Because the pharmacokinetics of cetuximab are well-known and the dosing is in the sub-therapeutic range, only limited pharmacokinetics will be performed (Tan, *et al*, 2006). Blood levels will be monitored before infusion (up to 30 days before) and after infusion (2 hours); on Day 1 as available; on Day 3 to 10; and Day 15 to 30. The half-life of cetuximab is known to be 4.5 days (package insert), however, single administration of low doses in humans results in significantly shorter half-life (Tan, *et al*, 2006). With a single dose at the levels proposed the half-life is expected to be approximately 2 to 3 days. Clearance of cetuximab occurs through the binding in the liver and through the reticuloendothelial system as occurs with endogenous immunoglobulin.

Prior to the test/loading dose, patients will be pre-medicated with an antihistamine (eg, diphenhydramine 50 mg IV) and any other medication as deemed appropriate by the treating physician prior to first dose. Treatment will be given in an infusion facility that routinely administers cetuximab and is equipped with emergency equipment and medication along with medical personnel in the event that the patient experiences a severe reaction.

If a subject experiences a clinically-important reaction to the test dose of unlabeled cetuximab, then the subject will be discontinued and withdrawn from the study, and will not receive the study drug Cetuximab-IRDye800. The test dose will be given according to the routine protocol for cetuximab infusion. The infusion 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 patient experiences a serious reaction that prevents receiving the entire test dose of cetuximab, the patient will be observed; but will not continue on-study to receive the study drug Cetuximab-IRDye800 . Any patient who does not receive the Cetuximab-IRDye800 will be considered a screen failure.

Administration of Cetuximab-IRDye800 will be performed consistent with institutional policy for administration of cetuximab. The solution will not be diluted or mixed with any other medications. Administration of Cetuximab-IRDye800 will occur by IV over 30 minutes to 1 hour. Patients will be observed for 3 hours after administration. Additional treatment for electrolyte imbalances will be given following institutional policy.

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

AEs will be graded according to the CTCAE v4.0 (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 Cetuximab-IRDye800
- Considered by the Investigator to clinically significant for patients who received Cetuximab-IRDye800. For this study, clinically significant is defined based on NCI definition – a result that is large enough to affect a patient's disease state in a manner that is noticeable to the patient and/or caregiver or requires intervention.
- Occurred within 15 days of receiving treatment with Cetuximab-IRDye800.

If a DLT is experienced, an additional 5 subjects will be added to that same dose level. The Stanford DSMC will be notified of all DLTs. The DSMC will evaluate safety data from all subjects through Day 15. If it is determined by the DSMC that the dosing is safe in the cohort where the DLT was experienced, the next higher dose level may be assigned and enrollment resumed.

4.4 Surgical Imaging

Tumor assessment. Please see previous section (3.0 Study Design) for more detail. The tumor will be imaged using the optical imaging systems. The primary tumor will be imaged prior to surgical resection, then the wound bed will be imaged after surgical resection, and then the primary tumor and frozen section margins imaged on 'back table' prior to sending to pathology. The information obtained from the imaging device or the images itself 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. Any additional lymphadenectomy specimen will be imaged prior to pathological analysis. Suspected positive nodes will be tagged with a surgical clip for correlation with positivity in pathological review. Once the specimens have been delivered to pathology, additional imaging will be performed as described above.

Imaging of the tumor specimens in situ will allow for calculation of tumor to background ratio (TBR). The fluorescence intensity of the tumor is compared to the grossly normal tissue imaged within the field and provides an assessment of Cetuximab-IRDye800 uptake within the tumor compared to normal tissues. Multiple measurements can be obtained at the time of surgery from still images and histograms for regions generated using ImageJ (NIH) as we have previously described (Heath, *et al*).

4.5 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.

4.6 Criteria for Removal from Study

Treatment will be stopped and patients will be removed from study due to unacceptable AEs, or withdrawal of consent.

4.7 Alternatives and Risk Protection / Mitigation

Alternatives

- This study is in the setting of tumor excision of biopsy-confirmed diagnosis of HNSCC, and the experimental component is infusion of Cetuximab-IRDye800, which may help visualize the tumor and tumor margins for the surgeon. The surgery itself is considered regular medical care. Therefore, the alternative to participating in this study is to have the tumor excision surgery without the Cetuximab-IRDye800 infusion.

Procedures to protecting against and minimize potential risks:

- This study is designed enroll appropriate patients populations (see Section 3.1 Inclusion Criteria, and Section 3.2 Exclusion Criteria).
- Subjects will be monitored regularly by the investigators and the study team after investigational Cetuximab-IRDye800 and surgery, through 30 days (± 1 week) post-Cetuximab-IRDye800 treatment, for AEs by means of physical exams, blood tests, and scans.
- 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

Cetuximab (Erbitux) is a recombinant human/mouse chimeric monoclonal antibody that binds to the extracellular domain of the human EGFR and functions as an antagonist by competitively inhibiting binding of Epidermal Growth Factor. Cetuximab is composed of the Fv regions of the murine anti-EGFR antibody with the human igG1 heavy and kappa light chain constant regions. The molecular weight of this recombinant antibody is approximately 152,000 kDa. Cetuximab is produced in murine myeloma cell culture and is supplied as a sterile solution at 2.0 mg/mL. The buffered solvent is 8.48 mg/mL sodium chloride, 1.88 mg/mL sodium phosphate dibasic heptahydrate, and 0.41 mg/mL sodium phosphate monobasic monohydrate without preservatives and with a pH between 7.0 and 7.4.

Cetuximab has been FDA-approved since 2006 for use in combination with radiation therapy for treatment of locally or regionally advanced squamous cell carcinoma. Cetuximab has also been approved for monotherapy or in combination with chemotherapeutic agents in colorectal cancer since 2004. As a result of almost a decade of use, its properties are well described, including its toxicity profile. Adverse reactions primarily include infusion reactions (20%), cutaneous (rash 90% and dry skin/pruritus 40 to 50%), CNS (fatigue 90% and headache 33%), hypomagnesium (55%), and gastrointestinal (abdominal pain 60%). Many of the reported AEs are associated with repeat administration rather than single dose as proposed in the current study. Infusion reactions are the most likely AE to be encountered in this study from cetuximab alone. We propose a test/loading dose of unlabeled cetuximab in order to determine if the subject has hypersensitivity to the unmodified monoclonal antibody.

The mean half-life of cetuximab is 112 hours and appears to plateau at does greater than 200 mg/m^2. Although the half-life of cetuximab when given in multiple doses is known to be 112 hours (4.7 days; cetuximab package insert), the half-life of cetuximab has non-linear kinetics and is significantly shorter when administered as a single dose. Studies have shown that when administered to humans at 50 mg/m^2, measurable concentrations of cetuximab could not be detected beyond 96 hours (4 days) and when administered at 100 mg/m^2 measurable concentrations of cetuximab could not be detected beyond 168 hours (7 days) (Tan, *et al*, 2006).

IRDye800CW

IRDye® 800CW 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 1:1 solution of Phosphate Buffered Saline (PBS): methanol has an absorbance maximum of 777 nm and an emission maximum of 791 nm. IRDye800CW is a near infrared fluorescent imaging dye that can be easily bound to proteins through the NHS Ester group (Figure 4) and has emission and absorption wavelengths as described in Figure 5. 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.

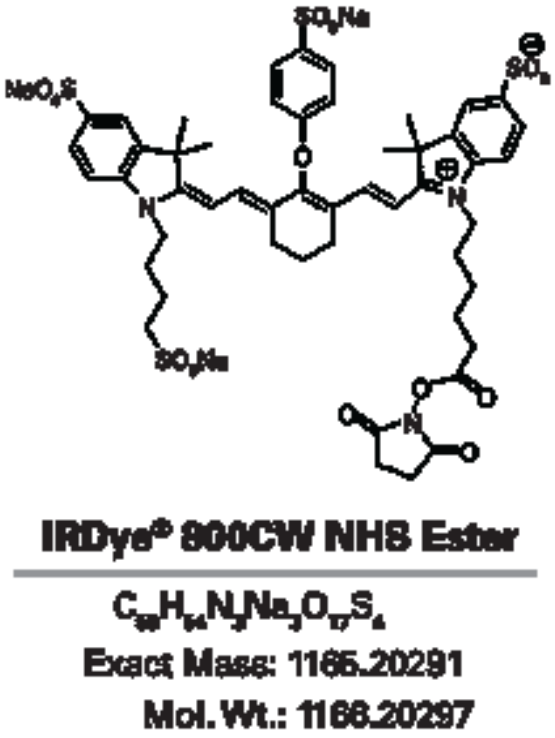

Figure 4. IRDye800CW NHS Ester. Molecular formula and molecular characteristics

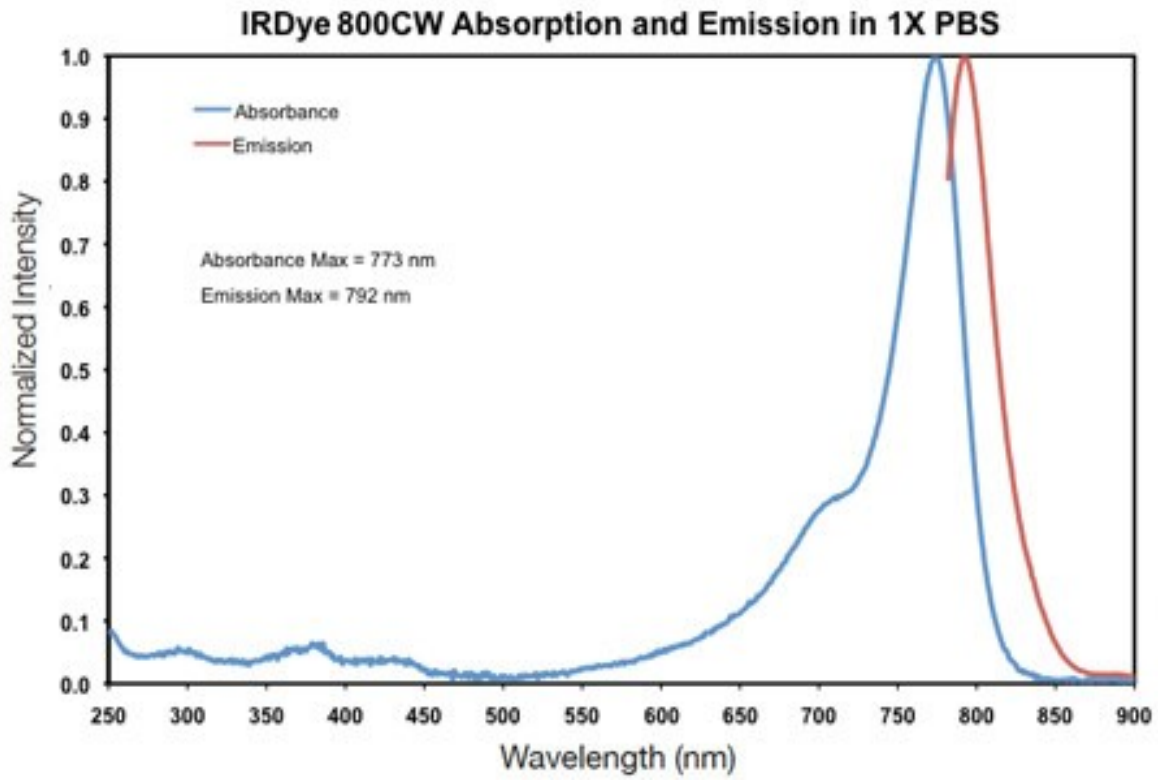

Figure 5. Absorption and emission spectra of IRDye800. Peak absorbance occurs at 773 nm and the peak emission is detected at 782 nm.

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 (Marshall, Draney, Sevick-Muraca, Olive). 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).

Cetuximab-IRDye800

Cetuximab-IRDye800 is produced by mixing the two components in phosphate buffer 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. Cetuximab-IRDye800 has been produced under cGMP conditions at University of Alabama at Birmingham using cGMP IRDye800CW and commercially available cetuximab. Formal testing of the Cetuximab-IRDye800 for stability, sterility and antigen specificity is contained within the CMC section according to an FDA approved process. The manufacturing and quality processes are documented elsewhere.

Cetuximab-IRDye800 was recently evaluated in a phase 1 clinical trial for surgical navigation in head and neck cancer at the University of Alabama by Dr Rosenthal (Rosenthal, *et al*, 2015).

12 subjects participated in a dose-escalation study, receiving up to 62.5 mg/m^2 Cetuximab-IRDye800. No grade 2 or higher AEs were attributed to Cetuximab-IRDye800. This maximum dose (62.5 mg/m^2) exceeds the dose that will be used in this study (50 or100 mg). There were very limited differences identified between cetuximab and Cetuximab-IRDye800, however there are several relevant findings:

1. The study demonstrated that the pharmacokinetics of single dosing with Cetuximab-IRDye800 are very similar to cetuximab; both have half-life of 2.6 days when dosed at 250 mg/m^2 in non-human primates. Therefore data will be collected using the known pharmacokinetics of cetuximab.
2. A small but statistically significant increase in the QTc interval was identified during the infusion of cetuximab or Cetuximab-IRDye800. Therefore patients at risk for arrhythmias associated with QT prolongation will be excluded (see exclusion criteria).
3. Phase 1 clinical trial data did not demonstrate a clinically significant Grade 2 reactions to Cetuximab-IRDye800 or was there a dose dependent relationship seen during the dose escalation of the study drug (Rosenthal, *et al*, 2015).

5.2 Expected Toxicities

With the exception of infusion reactions and allergic responses, the toxicities associated with cetuximab-based therapy are usually dose dependent and associated with prolonged therapy. Because the cetuximab will be delivered at between 1/100 to ¼ the therapeutic dose and will only be administered once, we anticipate the primary AEs will be related to the infusion reaction or allergic response.

5.2.1 Infusion Reaction

Because the study proposes to administer a single sub-therapeutic dose, the most likely AE we anticipate is an infusion reaction. Minor (Grade 1) infusion reactions from cetuximab can occur has high as 20%, with severe reactions (anaphylaxis) occurring in less than 1%. Serious infusion reactions can occur, requiring medical intervention and immediate, permanent discontinuation of cetuximab include rapid onset of airway obstruction (bronchospasm; stridor; and hoarseness); hypotension; shock; loss of consciousness; myocardial infarction; and/or cardiac arrest. Severe (NCI CTC Grades 3 and 4) infusion reactions occurred in 2 to 5% of 1373 study subjects receiving cetuximab, with fatal outcome in 1 subject.

5.2.2 Allergic Response

The Cetuximab-IRDye800 bioconjugate may provoke an allergic response since the humanized antibody has been modified. Although possible, we consider an allergic response unlikely because the agent is given as a single dose-limiting immunogenic potential (sensitization not possible) and similar antibody bioconjugates (radiolabeled antibodies) are given routinely without report of this complication. Blood will be collected for future analysis of antibodies that develop against the study drug.

5.2.3 Cardiac Toxicity

Cardiopulmonary arrest and/or sudden death occurred in 4 (2%) of 208 subjects treated with radiation therapy alone in a previous study. Three patients with prior history of

coronary artery disease died at home, with myocardial infarction as the presumed cause of death. This toxicity is not widely reported in other studies.

Prolonged QT interval: Preclinical studies in non-human primates in non-human primates identified an increase in the QTc interval for cetuximab and Cetuximab-IRDye800. There was also a statistically significant difference between the QTc interval between the cetuximab and Cetuximab-IRDye800 that will require assessment of interval ECG and exclusion criteria of patients at risk for arrhythmias, see exclusion criteria. Screening ECG and intermittent ECG data will be obtained as part of the study.

5.2.4 Pulmonary Toxicity

Interstitial lung disease (ILD) including 1 fatality has occurred in patients receiving cetuximab. Interrupt cetuximab for acute onset or worsening of pulmonary symptoms. Permanently discontinue for confirmed ILD.

5.2.5 Dermatologic Toxicity

Dermatologic toxicities, including acneiform rash; skin drying and fissuring; paronychial inflammation; infectious sequelae (eg, *S aureus* sepsis; abscess formation; cellulitis; blepharitis; conjunctivitis; keratitis/ulcerative keratitis with decreased visual acuity; cheilitis); and hypertrichosis occurred in patients receiving cetuximab. Severe acneiform rash occurred in 1 to 17% of patients.

Acneiform rash usually developed within the first two weeks of therapy and resolved in a majority of the patients after cessation of treatment, although in nearly half, the event continued beyond 28 days. Monitor patients receiving cetuximab for dermatologic toxicities and infectious sequelae. Patients should be instructed to limit sun exposure during cetuximab therapy.

5.2.6 Hypomagnesemia and Electrolyte Abnormalities

Hypomagnesemia occurred in patients receiving cetuximab, especially when given in combination with chemotherapy. The onset of hypomagnesemia and accompanying electrolyte abnormalities occurred days to months after initiation of cetuximab. Periodically monitor patients for hypomagnesemia, hypocalcaemia, and hypokalemia, during and for at least 48 to 72 hours following the completion of cetuximab. Replete electrolytes as necessary using guidelines in Section 4.2.2.

5.2.7 Other Toxicities

It is also possible that other cetuximab 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 (Marshall, *et al*), and it has similar chemical structure as indocyanine green (which has been delivered to humans in gram quantities for decades without significant toxicity.

5.3 Study Drug Discontinuation / Study Withdrawal

Study drug will be discontinued for any of the following:

1. Subject withdraws consent for the study
2. Death attributed to study drug
3. Serious systemic anaphylactic reaction (Grade 3)
4. Any serious rare reactions attributed to study drug
5. Subject experiences a serious reaction to the test/loading of cetuximab (ie, unable to continue and receive study drug Cetuximab-IRDye800). Because subjects who discontinue pursuant to this clause will not have received Cetuximab-IRDye800, these subjects will NOT be counted towards the accrual target defined at 12.1 Statistical Design / Sample Size.

If one of these events occurs, and is a serious adverse event (SAE, ie, 2, 3, or 4 above), the FDA, IRB and Stanford DSMC will be notified immediately and there will be a comprehensive review of the safety data prior to resuming and further study drug administration.

5.4 Availability

Unlabeled cetuximab will be sourced commercially

Cetuximab-IRDye800 has been produced under cGMP conditions at University of Alabama at Birmingham using cGMP IRDye800CW and commercially available cetuximab. Formal testing of the Cetuximab-IRDye800 for stability, sterility and antigen specificity is contained within the CMC section according to an FDA approved process. The manufacturing and quality processes are documented elsewhere.

5.5 Agent Ordering

University of Alabama at Birmingham will ship the Cetuximab-IRDye800 to be stored at the Stanford Investigational pharmacy under a material transfer agreement. Investigational pharmacy will be responsible for storage and dispensing of the drug following a standard institutional process after the patient is screen and enrolled on the study. Sufficient amounts of the Cetuximab-IRDye800 are available for this clinical trial.

The unlabeled cetuximab will dispensed by the investigational pharmacy to the study.

5.6 Agent Accountability

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

6. DOSE MODIFICATIONS

Apart from dose escalation already discussed in Section 4.3 there are no other dose modification 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 Sponsor and treated as a SAE with regard to the reporting time line. 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 must be collected.

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 v4.0) (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 of Adverse Events

AEs will be graded according to CTCAE v4.03. Both serious and non-serious AEs will be clearly noted in source documentation and listed on study-specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each AE to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. All SAEs will be tracked until resolution, or until 30 after the last dose of the study treatment.

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

All AEs, regardless of seriousness or relationship to study drug, including those occurring during the Screening period (where applicable), are to be 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 10 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.

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.

7.5.2 Recording and Reporting Adverse Events and Serious Adverse Events

It will be the responsibility of the Investigator to provide to the FDA all information that is concerning for significant hazards, contraindications, side effects or precautions felt significant to the safety of the drug being studied. 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.

All SAEs must be reported immediately (within 24 hours) from the time the Investigator learns of the event to the medical monitor. All SAEs and deaths must be reported in this fashion regardless of their causality assessment to the study drug.

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In the event of a SAE, the Investigator must report 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.

All SAEs (Grade 3 or above as graded by CTCAE v4.03) will be reported to the overall PI of the study at Stanford in writing within 5 business days of the event; Stanford will promptly report SAEs to CCTO for forwarding as necessary to the Stanford IRB and DSMC.

All SAEs must be reported to the FDA and IRB according to regulatory requirements.

Any woman who becomes pregnant or suspects she is pregnant while participating in the study must inform her treating physician and the Investigator immediately. The pregnancy must be followed through delivery or final outcome. This will also be true for any male who learn they may have impregnated a female while participating in the trial.

8. CORRELATIVE/SPECIAL STUDIES

Correlative studies will depend upon initial 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 OF SURGERY (DAY 2-5)	DAY 10 (±4 DAYS [12])	DAY 30 (±1 WEEK [12])
INFORMED CONSENT	X				
MEDICAL HISTORY	X				
PHYSICAL EXAMINATION [1]	X	X		X	X
VITAL SIGNS	X	X	X	X	X
PERFORMANCE STATUS [1]	X	X		X	X
ECG	X [2]	X [2]			X
CHEMISTRIES [3]	X	X	X		X
HEMATOLOGY [4]	X				X
TSH	X				
PTT/PT/INR	X				
SERUM PREGNANCY [13]	X	X [13]			
PK - FLUORESCENT-LABELED ANTIBODY [5]		X	X	X	X
PK-IMMUNOGENICITY [6]		X			X
IV INFUSION OF CETUXIMAB (100 MG LOADING DOSE) [7]		X			
IV INFUSION OF CETUXIMAB-IRDye800 [8] **(AFTER COMPLETION OF TEST DOSE)**		X			
SURGICAL RESECTION			X		
TUMOR IMAGING			X [9]		
PATHOLOGICAL EVALUATIONS [10]			X		
ADVERSE EVENTS		X	X	X	X
CONCURRENT MEDICATIONS	X	X	X	X	X

1. A physical examination and performance status assessment will be done at Screening; Day 0; ; Day 6 to14; and Day 23 to37. Examinations collected for surgical procedure are acceptable for screening purposes. Examinations do not have to be repeated on Day 0 if completed within 14 days of infusion.
2. ECG will be obtained at screening to evaluate baseline and then at 30 minutes and 2 to 3 hours after initiation study drug infusion. A previous ECG that was obtained as part of standard of care may be used as the baseline ECG as long as it was obtained within 30 days of the start of treatment.
3. Chemistries include: Na+; K+; CO_2; Cl; Mg++; Ca++; BUN; Cr; Phosphorus; albumin; alkaline phosphatase; ALT; AST; total bilirubin; total protein. Labs drawn as part of the subject's standard of care for surgery may be used for screening. Chemistries do not have to be repeated on Day 0 if completed within 14 days of infusion.
4. Hematology values include CBC and platelet count. Labs drawn as part of the subject's standard of care for surgery may be used as the baseline hematology values as long as they are within two weeks of treatment.

5. Serum level of cextuximab-IRDye800 will be drawn at baseline prior to loading dose; 2 hours after administration; Day 6 to14 and Day 23 to37to assess circulating fluorescent-labeled antibody and unconjugated dye. This time point will be Day 23 to37, but will be abbreviated as 15 days throughout the protocol.
6. Blood will be obtained pre-infusion and at 30 days after infusion and banked for future immunogenicity testing.
7. To be given over 30 minutes followed by a 30 minute observation period prior to administration of the labeled Cetuximab. Subjects will continue on-study if they successfully receive the loading/test dose.
8. Infusion of Cetuximab-IRDye800 to be given over one hour followed by a three hour observation period.
9. Tumors will be imaged intraoperatively using the optical imaging devices Novadaq SPY/LUNA; IR9000 Fluorescence Imaging System with Open-Field Handheld Fluorescence Imaging Camera and/or Pinpoint Endoscopic Fluorescence Imaging Camera; or SurgVision Explorer Air multi-spectral fluorescence reflectance system to determine threshold of detection. Surgery is performed 2 to 5 days after infusion of study drug.
10. All resected tissues will be evaluated for NIR fluorescence and photoacoustic signal prior to pathological examination.
11. The Day 1 visit will be performed based on subject availability.
12. Based on the subject's standard of care admission or return visit
13. Serum pregnancy test will be obtained on all women of childbearing age at baseline; a urine pregnancy test will be performed on Day 0 if the result of a serum pregnancy test within 72 hours before administration of Cetuximab-IRDye800 is not available.

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
- Assessment of performance status
- Physical examination
- Vital signs to include blood pressure; heart rate; respiratory rate; temperature
- Height and weight
- Chemistries (electrolytes; total bilirubin; ALT; AST; alkaline phosphatase; serum albumin; creatinine; BUN; magnesium; phosphorus). Labs drawn as part of the subject's standard of care for surgery may be used as the baseline chemistries as long as they are within two weeks of treatment.
- CBC and platelets – labs drawn as part of the subject's standard of care for surgery may be used as the baseline values as long as they are within two weeks of treatment
- TSH
- PTT/PT/INR – labs drawn as part of the subject's standard care for surgery may be used as the baseline values as long as they are within two weeks of treatment
- Serum pregnancy test for women of childbearing potential
- ECG

9.2.2 Day 0

- Physical examination (within 14 days)
- Vital signs to include blood pressure; heart rate; respiratory rate; temperature
- Performance status (within 14 days)
- Chemistries (electrolytes; total bilirubin; ALT; AST; alkaline phosphatase; serum albumin; creatinine; BUN; magnesium; phosphorus; within 14 days)

- Pregnancy test for women of childbearing potential. If the result of a serum pregnancy test within 72 hours before administration of Cetuximab-IRDye800 is not available, a urine pregnancy test will be performed on Day 0
- Blood sample for Cetuximab-IRDye800 antibody prior to infusion
- Blood sample for future assessment of immunogenicity
- **IV infusion of cetuximab (100 mg) over 30 minutes**
    - Vital signs (blood pressure; heart rate; respiratory rate; temperature) during infusion at 15 minutes and 30 minutes
    - Observation for 30 minutes
    - Vital signs (blood pressure; heart rate; respiratory rate; temperature) post-infusion at 15 minutes and 30 minutes
    - Assessment for AEs
    - Assessment for concomitant medications
    - ECG will be obtained 30 minutes post-initiation of loading dose
- **Post-cetuximab infusion observation period of 30 minutes**
- **IV infusion of Cetuximab-IRDye800 over 30 minutes to 1 hour**
    - Vital signs (blood pressure; heart rate; respiratory rate; temperature) during infusion at 15 minutes; 30 minutes; and 60 minutes
    - Observation for 2 to 3 hours
    - Vital signs (blood pressure; heart rate; respiratory rate; temperature) post-infusion at 15 minutes; 30 minutes; 60 minutes; 90 minutes; 2 hours; and prior to discharge
    - Blood sample for Cetuximab-IRDye800 antibody 2 hours after completion of infusion
    - Assessment for AEs
    - Assessment for concomitant medications
    - ECG will be obtained 2 to 3 hours post-initiation of the study drug

9.2.3 Day 2-5 (Day of Surgery)

- Vital signs to include blood pressure; heart rate; respiratory rate; temperature prior to surgery
- Chemistries (electrolytes; total bilirubin; ALT; AST; alkaline phosphatase; serum albumin; creatinine; BUN; magnesium; phosphorus)
- Blood sample for fluorescent-labeled antibody
- Scheduled routine surgical resection
- Intraoperative tumor imaging
- Pathological evaluations
- Assessment for AEs
- Assessment for concomitant medications

9.2.4 Day 10 (+/- 4 days)

- Physical examination
- Vital signs to include blood pressure; heart rate; respiratory rate; temperature
- Performance status
- Blood sample for fluorescent-labeled antibody

- Assessment for AEs
- Assessment for concomitant medications

9.2.5 <u>Day 30 (+/- 1 week)</u>

- Physical examination
- Vital signs to include blood pressure; heart rate; respiratory rate; temperature
- Performance status
- Chemistries (electrolytes; total bilirubin; ALT; AST; alkaline phosphatase; serum albumin; creatinine; BUN; magnesium; phosphorus)
- CBC and platelets
- Assessment for AEs
- Assessment for concomitant medications
- Obtain blood sample for fluorescent-labeled antibody and future assessment of immunogenicity.
- ECG

9.3 Description of Evaluations / Procedures

9.3.1 Laboratory Evaluations: Blood specimens will be collected for CBC; platelets; electrolytes; total bilirubin; ALT; AST; alkaline phosphatase; serum albumin; creatinine; BUN; magnesium and phosphorus; PTT/PT/INR 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 abdominal 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; at Day 0 during and after the infusion; and then at Day 30. QT/QTc interval will be assessed for each time point.

10. MEASUREMENTS

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance

10.1 Primary Outcome Measure

The primary objective of the study is to determine the efficacy of Cetuximab-IRDye800 to identify cancer compared to surrounding normal tissue

Outcome measure: Tumor to background ratio (TBR) and signal-to-noise ratio (SNR). Fluorescence and photoacoustic intensity of tumor tissue compared to that of normal surrounding tissue.

10.2 Secondary Outcome Measure

The secondary objectives are to determine the safety profile and tolerability of Cetuximab conjugated to the optical dye, IRDye800CW (Cetuximab-IRDye800).

- **Outcome measure**: Number of Grade 2 or higher AEs which have been determined to be clinically significant and definitely, probably or possibly related
- **Time Frame**: Safety events will be recorded over the 30-day observation period
- **Safety Issue**: Yes

11. MULTISITE REGULATORY CONSIDERATIONS

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring board for this study, please refer to the Data and Safety Monitoring Committee SOP for more information.

11.1 Monitoring plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study

11.2 Role of the Stanford Data and Safety Monitoring Committee (DSMC)

The Stanford DSMC will have the following roles in this study:

- The DSMC will audit study-related activities approximately once per year 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 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 review SAEs and protocol deviations from all sites 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.
- If Cetuximab-IRDye800 is discontinued at any clinical site, further dosing will be suspended and the DSMC will be notified of the SAE within 24 hours. The DSMC will conduct a comprehensive safety review of the safety data. On approval of the DSMC, the study may resume at that dose level, or at a lower dose level determined by the DSMC to be the probable maximum tolerated dose (MTD).
- If a DLT (see Section 5.2.4) occurs, the Stanford DSMC will be notified of the DLT. An additional five subjects will be added to that same dose level.
    - At any dose level identified by the DSMC as the probable MTD, an additional five subjects may be enrolled at the probable MTD dose level, so that a minimum of 10 subjects is evaluated at that dose level.

11.3 Protocol Review and Amendments

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 Protocol Director will disseminate the protocol amendment to all participating investigators. Investigators will be expected to obtain IRB approval within 90 days for all amendments.

11.4 Data management

The research coordinator at Stanford will be responsible for database records of subject data. The data will be kept in a database, under password protection with access limited to specific areas of the database. A chart with all of the relevant research subject information will be maintained for each subject at each institution by the research coordinator for that specific institution. Subject charts may be reviewed by Stanford PI and Study Coordinator for yearly audits.

11.5 Study Documentation

The Protocol Director and participating site investigators 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. Stanford University will enter all the study related data in the OnCore portal.

The principal investigator will be responsible for maintaining the clinical protocol at Stanford University and subjects' study charts; for reporting AEs that occur at UAB; assuring that consent for subjects is obtained and documented; and for obtaining and maintaining IRB approval at Stanford University. The principal investigator will report all continuing renewals received for the study to the Sponsor / Stanford Protocol Director.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

The primary endpoint will be evaluated in the following 4 ways:

In Vivo Fluorescent Imaging. When imaging the tumor in vivo we will calculate the tumor-to-background ratio (TBR). The fluorescence intensity of the tumor is compared to the grossly normal tissue imaged within the field and provides an assessment of Cetuximab-IRDye800 uptake within the tumor compared to normal tissues. Multiple measurements can be obtained at the time of surgery from still images and histograms for regions will be generated using ImageJ (NIH) as we have previously described (Andrades, *et al*, 2008; Day, Beck, Deep, *et al*, 2013; Day, Beck, Heath, *et al*, 2013; Day, Sweeny, *et al*, 2013; Heath, *et al*.).

Assessment of Regional Lymphatics: Imaging results (categorical positive/negative) will be correlated with histopathological evidence of disease.

Ex Vivo Fluorescent Imaging. The variables will be correlated with tumor fluorescence parameters. Sensitivity, specificity, positive predictive value and negative predictive value will be determined as previously described by us (Rosenthal, *et al*, 2006; Rosenthal, *et al*, 2007). The linear relationship between fluorescence and tumor size will be computed as Spearman's correlation coefficient.

Pathological Correlates: To determine biological characteristics associated with fluorescence intensity, paraffin embedded blocks from the tumor will be obtained after routine pathologic analysis and will undergo analysis for: 1) size of the epithelial tumor compartment in percent of total, 2) tumor viability, and 3) EGFR immunohistochemistry (Gleysteen, *et al*, 2007b; Kulbersh, *et al*, 2007; Rosenthal, *et al*, 2006; Rosenthal, *et al*, 2007; Withrow, *et al*, 2007).

Ex Vivo Photoacoustic Imaging (PAI). The average signal intensity (or more specifically the signal-to-noise ratio measured in dB) of the tumor will be calculated in the region of interest (ROI) and compared to normal surrounding tissue.

Direct Comparison of Fluorescent and Photoacoustic Imaging.

The number and location of detected lesions by fluorescent and photoacoustic techniques will be recorded on a schematic case-report form (CRF). This will be done to determine if both imaging techniques are complementary to each other and thereby of synergistic advantage for pancreatic cancer surgery.

The secondary endpoint of the study is to determine the tolerability of cetuximab conjugated to the optical dye, IRDye800CW (Cetuximab-IRDye800). The safety endpoints are type, incidence, severity, seriousness, and study treatment relatedness of AEs. Descriptive statistical analysis of subject disposition, baseline characteristics, exposure to study drug, and AEs will be performed. Descriptive statistics for continuous data will include mean, standard deviation,

median, minimum, and maximum values. Frequencies and percentages will be used to summarize categorical data.

Sample size. We propose to enroll and treat 10 subjects with cetuximab-IRDye800 in this study. These 10 subjects will be assigned in two cohorts. Cohort 1 (first 5 subjects) will receive a 100 mg unlabeled test/loading dose of cetuximab and 50 mg of cetuximab-IRye800. Cohort 2 (the last 5 subjects) will receive a 100 mg unlabeled test/loading dose of cetuximab and 100 mg of cetuximab-IRDye800. The rationale for these doses is based on preliminary results for our phase 1 clinical trial (Rosenthal, *et al*, 2015) which demonstrated that: (a) 50 mg dose provides the highest tumor-to-background ratio compared to doses higher and lower, and (b) the 100 mg unlabeled test/loading dose is optimal in reducing normal tumor background. Subjects who experience a serious reaction to the test dose of unlabeled cetuximab will not continue and receive Cetuximab-IRDye800, and will not be counted towards the study accrual target. Subjects who fail the cetuximab test dose will be accounted separately from the study cohorts.

Animal models demonstrate a sensitivity of 92% and specificity of 100% using Cetuximab-IRDye800 (Kulbersh, *et al*, 2007). We expect that for each subject approximately 10-12 frozen sections will be obtained from the wound bed with a positive rate of 25% (Cook, Jones, Phillips, Soler Lluch, 1993; McMahon, *et al*, 2003; Woolgar Triantafyllou, 2005)

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APPENDICES

APPENDIX A: Participant Eligibility Checklist

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

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

Protocol Title:	Intraoperative Pancreatic Cancer Detection Using Multimodality Molecular Imaging
Protocol Number:	IRB-35789 / PANC0024
Principal Investigator:	George Poultsides, MD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC-approved ☒ IRB-approved ☒ Contract signed ☒

III. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB-approved protocol)	**Yes**	**No**	Supporting Documentation*
1. Biopsy-confirmed or suspected diagnosis of pancreatic adenocarcinoma	☐	☐	
2. Subjects diagnosed with any T stage that are scheduled to undergo surgical resection. Subjects with metastatic disease or a new primary will be allowed	☐	☐	
3. Planned standard of care surgery with curative intent for pancreatic cancer	☐	☐	
4. Age ≥ 19 years	☐	☐	
5. Life expectancy of more than 12 weeks	☐	☐	
6. Karnofsky performance status of at least 70% or ECOG/Zubrod level 1	☐	☐	
7. Hemoglobin ≥ 9 gm/dL	☐	☐	
8. Platelet count ≥ 100,000/mm^3	☐	☐	
9. Magnesium, potassium and calcium > the lower limit of normal per institution normal lab values	☐	☐	
10. TSH < 13 micro International Units/mL	☐	☐	

Exclusion Criteria (From IRB-approved protocol)	**Yes**	**No**	Supporting Documentation*
1. Received an investigational drug within 30 days prior to first dose of Cetuximab-IRDye800	☐	☐	
2. Myocardial infarction (MI); cerebrovascular accident (CVA); uncontrolled congestive heart failure (CHF); significant liver disease; or unstable angina within 6 months prior to enrollment	☐	☐	
3. History of infusion reactions to cetuximab or other monoclonal antibody therapies	☐	☐	
4. Pregnant or breastfeeding	☐	☐	
5. Evidence of QT prolongation on pretreatment ECG (greater than 440 ms in males or greater than 450 ms in females)	☐	☐	
6. Lab values that in the opinion of the primary surgeon would prevent surgical resection	☐	☐	
7. Subjects receiving Class IA (quinidine, procanamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents	☐	☐	

*****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: IR9000 Fluorescence Imaging System, Novadaq

The system is based on Novadaq's commercial PINPOINT Endoscopic Fluorescence Imaging System (PC9000). It is an in development complimentary product to the PINPOINT for open field imaging. There are two configurations: the Endoscopic Fluorescence Imaging System for IR (EFI-IR) and the Handheld Fluorescence Imaging System for IR (HH-IR).

The EFI-IR is very similar to the commercial PINPOINT system with the following modifications:

1. Different laser source and excitation wavelength in the Video Processor/Illuminator (VPI).
    a. Part of development is ensuring the new laser meets the 3R requirement for laser safety (as PC9000 does)
    b. 60601-1 Electrical Medical device safety compliance is assessed. PINPOINT is 60601-1 and EMC certified. Leakage currents, surface temperature and hipot testing are run in house in accordance to the test methods prescribed in the IEC 60601 standards to ensure continued compliance.
2. An additional filter within the endoscopic camera.
    a. This is a passive part that does not affect safety.
    b. Camera is sterilizable by Sterrad.

The other components of the EFI-IR system are identical to the PC9000 system including cart, medical grade monitor, medical video recorder, sterilizable light guide, and endoscopes.

The handheld open field configuration, HH-IR, uses the same VPI as the EFI-IR. The imaging head is a handheld unit containing the camera optics and sensor, and the illumination optics. Similarly to the EFI-IR system the HH-IR system will be assessed for Electrical Medical Device Safety and EMC compliance in comparison to an equivalent device that is currently undergoing certification. The imaging head will be compatible with the ND3000, a sterile drape that is approved for use in the US with the SPY2000 system.

APPENDIX C: Explorer Air, SurgVision

Investigational Device

The SurgVision Explorer Air (beta version) is a multi-spectral fluorescence camera, which is specifically-designed to detect fluorescence in human tissue with the highest sensitivity. A schematic overview is shown in Figure 1. It consists of a camera head mounted to an adjustable arm and a medical cart carrying all hardware and the arm. During surgery, the camera head is placed above the field of interest to detect and image fluorescence signals. The camera operates at frame rates of 56 fps, which allows for imaging in real-time. Due to the 2-channel set-up accommodating a color channel and a fluorescence channel, the user can view the color image, the fluorescence image and the co-registered overlay image at the same time. The technology has been successfully used in clinical trials (NCT01508572; NCT02583568; NL45588; NCT01972373; NCT02129933; NCT02113202) and a risk assessment used to receive respective IRB approvals is available on demand. The technical specifications of the SurgVision Explorer Air (beta version) are as follows:

Physical dimensions	
Height x Width x Length	155 x 90 x 65 cm
Weight	90 kg
Power	
Input Voltage	240 V 50 Hz
Power consumption	Approx. 1500 W
Optical Properties OR use	
Focal Length Lens	16 mm
F number	1.6
Working range	15 to 50 cm
Field of view	15 cm
Fluorescence excitation	
Wavelength	760 nm
Output power	Max 2.6 W
Visible light	
Luminous flux	Approx. 1,100[2]
Color temperature	Approx. 5,600 K
Fluorescence camera	
Active Pixels	512 x 512
Pixel Size	16 x 16 µm
Image Area	8.2 x 8.2 mm
Well Depth	180,000 e-
Max. Readout Rate	17 MHz
Frame Rate	Max 56 fps
Read Noise	< 1 e- with EM gain
QE max	> 90%
Cooling	Water and/or forced ventilation water cooling unit included
Color Camera	

Resolution	2046 x 2046 (H x V pixels)
Pixel Size horizontal/vertical	5.5 x 5.5 µm
Frame Rate	25 fps
Mono/Color	Color
Sensor Size	11.26 x 11.26 mm
Image processing	
Operating system	Microsoft Windows
File format images	Fits
Camera Arm	
Arm extension	1100 mm
Height adjustment	900 mm

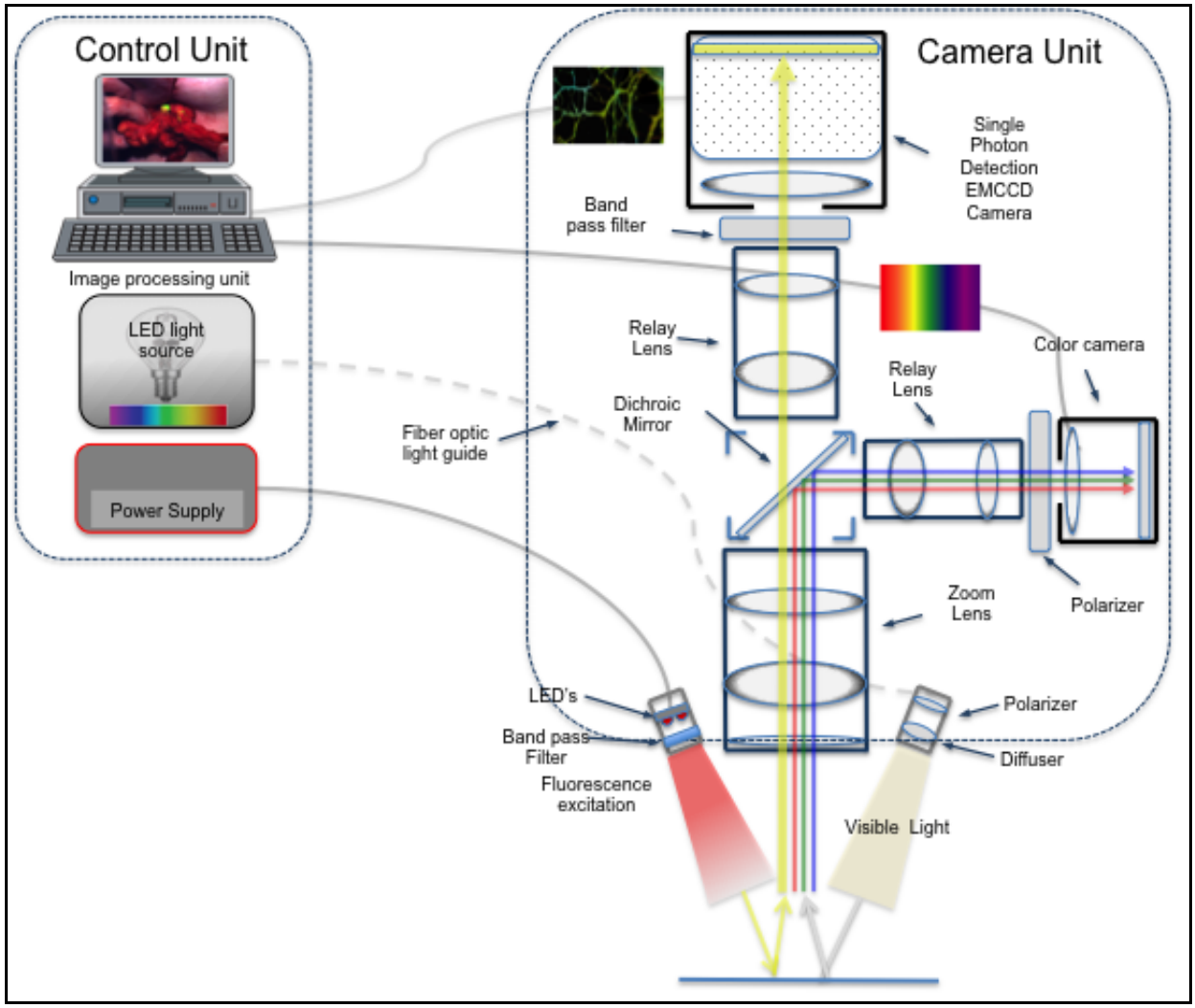


Figure Appendix 1. Schematic drawing of the SurgVision Explorer Air (beta version)

Determination of Significant Risk or Non-Significant Risk

The proposed SurgVision Explorer Air (beta version) is an investigational device. When evaluated as per 21 CFR § 812.3(m) - lnvestigational Device Exemptions, Definitions, the system does not present a potential for serious risk to the health, safety, or welfare of a subject. Specifically,

- The device is not intended as an implant and does not present a potential for serious risk to the health, safety, or welfare of a subject.
- The device is not purported or represented to be for a use in supporting or sustaining human life and does not present a potential for serious risk to the health, safety or welfare of a subject.
- The device is not for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.
- The device does not otherwise present a potential for serious risk to the health, safety or welfare of a subject.

The investigational device is consequently classified as non-significant risk in the context of the proposed investigational studies and, under 21 CFR § 812.2(b), an investigational Device Exemption application is not required.

APPENDIX D: Photoacoustic imaging

Rationale

The use of molecular imaging has been shown to add relevant information to the surgeon in decision-making process during surgery and **there is a need for non-ionizing imaging technologies that reliably visualize cancer with enhanced sensitivity and specificity.** The molecular imaging techniques that are suitable for intra-operative use are photoacoustic and fluorescence imaging. The main advantage of photoacoustic imaging (PAI) is providing images at clinically relevant depths (5-7cm) with high spatial resolution (Zackrisson, van de Ven, *et al.* 2014). Which is essential for determination of the resection margin (> 1 cm) to be able to generate tumor-free margins. Fluorescent imaging on the other hand is superior for imaging superficial lesions and vital structures (Vahrmeijer, Hutteman, *et al.* 2013).

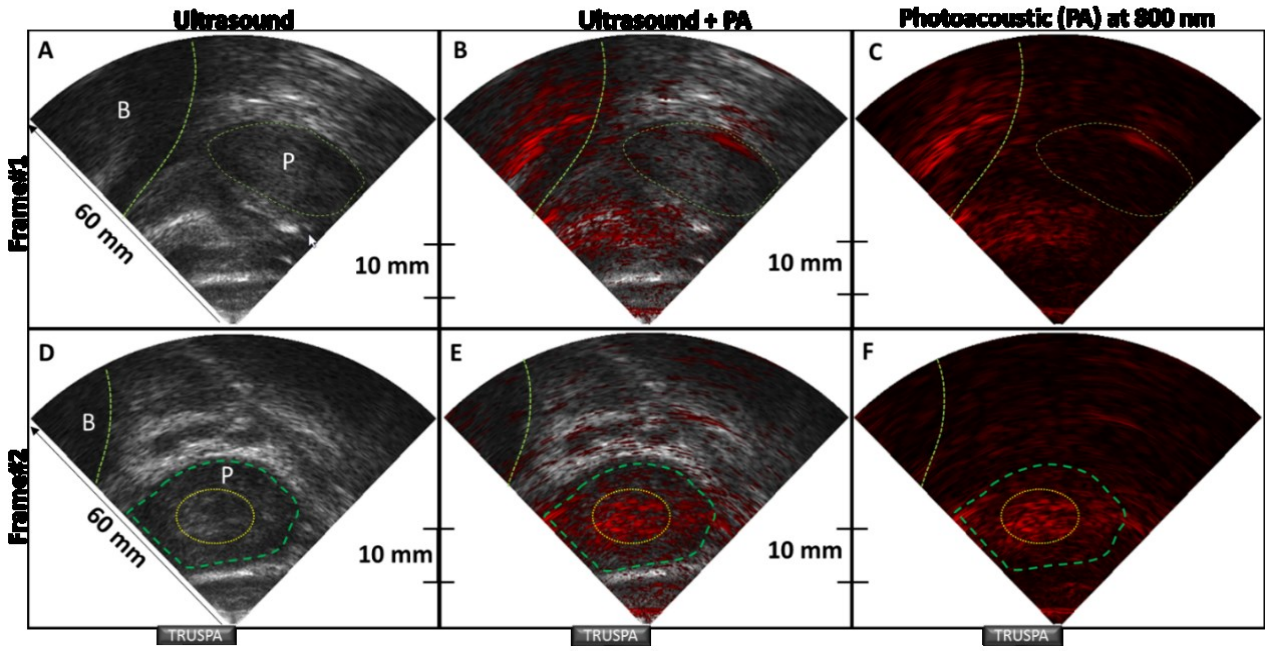
The principles of photoacoustics

Alternative imaging techniques not involving ionizing radiation, e.g. optical imaging, have emerged during the last decades. Conventional optical imaging devices shine light on tissues. This light is both absorbed and scattered by the different tissue components. NIR wavelength range of 600-1000 nm is used to obtain sufficient tissue penetration. After passing through the tissue the remaining light is registered by detectors and an image can be reconstructed with the help of computer algorithms.

In PAI, a pulsed nano-second laser beam illuminates the tissue sample and propagates diffusely inside the biological tissue. When the light pulses are absorbed by certain light absorbing molecules (such as the hemoglobin in the blood vessels, melanin in skin, water) present inside the tissue, wide band ultrasound (photoacoustic) waves are generated due to transient thermoelastic expansion. The waves are then detected by wide band ultrasonic transducer, Figure 3. PAI can image tissue using endogenous contrast agents such as hemoglobin or exogenous contrast agents, such as ICG, and IRDye800. Photoacoustic (PA) molecular imaging strategies using imaging agents targeting certain cancer biomarkers with high specificity and sensitivity can enhance and complement the functional imaging capabilities of the intrinsic signals (Gerling, Zhao, *et al*. 2014, Shao, Morgounova, *et al*. 2013).

Imaging device

PAI is an optical (non-ionizing) imaging technique that can provide high optical contrast images with the high spatial and temporal resolution of US. It can in real-time simultaneously provide structural, functional and molecular information of tissues, provide information on tumor characteristics. The technique has been evaluated in several phantom- and animal studies, and in a clinical pilot study in humans imaging the prostate with a hand held photoacoustic imaging system, Figure Appendix 1. At this point, this technique is used in a clinical trial with prostate cancer patients (NCT01551576),



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B: Bladder P: Prostate

Figure Appendix 1: (A-C) of Frame#1 and (D-F) of Frame#2 shows ultrasound, co-registered ultrasound and photoacoustic, and photoacoustic images of human prostate obtained with TRUSPA device. Green circled area is prostate region and yellow circled region showing abnormal photoacoustic contrast in the prostate is due to the presence of prostate tumor in the right base close to the rectal wall.

A hand held PAI instrument has been developed at Stanford (Kothapalli, *et al*, manuscript in preparation), Figure Appendix 2. The probe integrates a fiber optic light guide and a state of art two-dimensional 16x16 element capacitive micromachined ultrasound transducer (CMUT) arrays with a center frequency of 5.5 MHz. The PAI instrument has been tested in a deep tissue phantom model with optical absorbing objects of different absorption coefficients. It was possible to demonstrate three-dimensional (3-D) photoacoustic imaging of light absorbing objects embedded as deep as 5 cm inside strong optically scattering phantoms. In addition, the sensitivity of PAI to the concentration of indocyanine green (ICG) dye at 5cm depth inside the phantom was studied. Under optimized experimental conditions, the objects at 5 cm depth could be imaged with SNR of about 35 dB and a spatial resolution of approximately 500 μm. Preliminary results of our group also showed a good photoacoustic signal with the dye IRDye800 under experimental conditions.

For these specific experiments, the laser wavelength was at 800 nm and the laser power was 15.4 mJ/cm^2. This power is below the American National Standard for Safe Use of Lasers (ANSI) safety limit for human skin exposure of 20mJ/cm (Oraevsky AA, Ermilov, S, *et al*. 2006).

The device is considered a low risk device and an Investigational Device Exemption (IDE) has been approved by the IRB at Stanford.

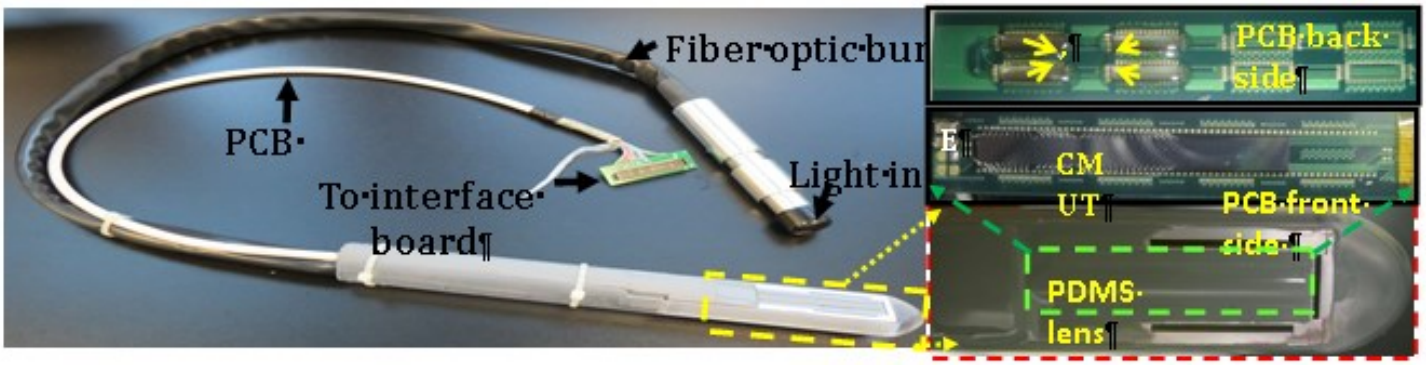

Figure Appendix 2. Image showing the hand held photoacoustic probe. IC and CMUT are flip-chip bonded and placed on PCB (printer circuit board). PCB is rested in between two parallel fiber optic light guides that focus light 0.5 inch above the CMUT surface. The entire PCB-light guide assembly is housed in acrylic probe that has similar dimensions as conventional ultrasound probe.

Photoacoustic imaging in Cetuximab-IRDye800 trial

Photoacoustic imaging will be done at the backtable in the imaging process. That means during the optical imaging of tumor and waste tissue during pathological processing at the back-table and **will not be used on subjects or in patient-care setting**. Once the tumor has been removed from the subject and sent to pathology, additional imaging will be performed on pathological specimens using the above described system. This will be done in the frozen section in collaboration with the Department of Pathology to ensure preservation of tumor related information. Normal waste tissues will also be imaged. Resected tumor tissue sent for laboratory analysis will be analyzed according to current SOPs, using approved products as applicable. Although this photoacoustic imaging device is not FDA approved, it will **not be involved in direct subject contact or interfere with standard of care pathological processing**. Data from the research use of the device will not be used for diagnosis or other medical decision making.

Availability

Stanford University provides the PAI device.