MELVIN AND BREN SIMON CANCER CENTER
INDIANA UNIVERSITY

INTRAOPERATIVE FOLATE TARGETED FLUORESCENCE IN RENAL CELL CARCINOMA

INDIANA UNIVERSITY PROTOCOL IUSCC-0546

Title: Intraoperative folate targeted fluorescence in renal cell carcinoma

Test Drug: OTL38: folate conjugated with a Tetrasodium salt solution (C61H63N9Na4O17S4, molecular weight 1414.42) (6 mg/vial) containing 2 mg/mL OTL38 free acid in 3 mL Water for Injection provided by On Target, LLC.

Sponsor/PI:
Chandru P. Sundaram, MD
Professor of Urology, Director of Residency Program and Minimally Invasive Surgery, Department of Urology
Indiana University
535 N Barnhill Dr, STE 420
Indianapolis, IN 46202
Phone 317.948.3098
Fax 317.944.0174
sundaram@iupui.edu

Co-investigators:
Steven Kheyfets, MD
George Sandusky, DVM, PhD

Support: On Target Laboratories, LLC

IND#: 127848

Date of Protocol: May 22, 2017

CONFIDENTIALITY

INVESTIGATOR'S AGREEMENT

By signing below I confirm that I have read this protocol and agree:

- To assume responsibility for the proper conduct of this study at this site
- To conduct the study according to the procedures described in this protocol and any future amendments
- Not to implement any deviation from, or changes to, the protocol without written approval from the Institutional Review Board (IRB), except where necessary to eliminate immediate hazard to the subject(s)
- That I am aware of all updates and will comply with all applicable regulations and guidelines

Principal investigator's signature

Date

Chandru P. Sundaram, MD

Principal investigator's name (print)

Professor of Urology, Director of Residency Program and Minimally Invasive Surgery, Department of Urology

Principal investigator's title (print)

PROTOCOL SYNOPSIS

Primary Objectives:	To explore the use of OTL38 and fluorescence imaging to visualize renal cell carcinoma (RCC) at the margins of resection in partial nephrectomy and in lymph node(s) or other metastases for radical nephrectomy.
Secondary Objectives:	To assess the specificity of tumor fluorescence obtained using OTL38 with folate receptor alpha positive (FRα+) renal cell carcinoma cancer lesions detected via immunohistochemistry To assess the agreement between fluorescence imaging using OTL38 and FRα by immunohistochemistry To assess the agreement between fluorescence imaging using OTL38 and visual/tactile assessments To assess inter-observer variation of tumor fluorescence images captured during surgery, physician rated usefulness of imaging, and safety of OTL38
Study Design:	This is a pilot, phase 2, non-randomized study in patients with RCC, scheduled to undergo primary, partial, or radical nephrectomy. The current study will have 2 separate arms, but the data from each arm will not be compared with each other. Arm 1: patients with partial nephrectomy for localized RCC Arm 2: patients with radical nephrectomy and lymph node dissection for locally advance or metastatic RCC
Test Drug/Dose:	OTL38 Injection: folate analog ligand conjugated with an indole cyanine-like green dye as a solution in vials containing 3 mL at 2 mg/mL provided by On Target, LLC. Single-dose 0.025 mg/kg administered IV, less than 2 hours prior to skin incision. Diphenhydramine 25 mg will be given prior to administering the study agent.
Duration of Treatment	Patients will receive a single dose of OTL38 approximately 2 hours before skin incision. Safety assessments will occur at follow-up phone visit at 10 days (±3 days) and follow-up office visit at 1 month (± 14 days)
Camera Systems	The da Vinci® Fluorescence Imaging Vision System manufactured by Intuitive Surgical, Inc. or the VSiii 3DHD system manufactured by Visionsense will be used in this study to visualize OTL38-labelled tumors during robotic partial and radical nephrectomies. The SPY Elite Imaging System manufactured by NOVADAQ will be used in open radical nephrectomies. These systems are not approved for use with OTL38, and are therefore considered investigational for purposes of this study. The da Vinci® Fluorescence Imaging Vision System has integrated fluoresence imaging capable of providing real-time, imaging identification of fluorescently tagged objects.

	The Visionsense VSiii system is a complete solution enabling surgeons to use a minimally invasive stereoscopic (3D) camera, for Neurosurgical, ENT, Arthroscopy (CE only), and Laparoscopy applications. The system has been successfully used by surgeons in a wide range of MIS procedures. The VSiii system has 510(k) clearance and is CE Mark approved. The SPY Elite Intraoperative Imaging System is a fluorescence imaging system that allows surgeons to capture, review, print and archive high-quality fluorescence images of near infrared (NIR) emitting imaging agents during surgical procedures. The positioning of the Imaging Head is controlled by the surgeon or physician. Image sequence capture is accomplished at operator's command, and images can be replayed immediately for review. When used in combination with indocyanine green, SPY Elite has indications for use that include • visual assessment of blood flow as an adjunctive method for the evaluation of tissue perfusion, and related tissue-transfer circulation in tissue and free flaps used in plastic, micro-, and reconstructive procedures • visual assessment of blood flow in vessels and related tissue perfusion during gastrointestinal surgical procedures • visual assessment of blood flow in vessels and related tissue perfusion during cardiovascular surgical procedures In all other surgical imaging applications – such as for use with targeted NIR imaging agent technologies – SPY Elite is designated as an investigational device.
Enrollment	Subject accrual will take up to 24 months.
Number of Subjects:	20 patients with RCC will be enrolled in this study. The study will consist of two arms studied simultaneously: • Arms 1: 10 patients with partial nephrectomy for localized RCC • Arms 2: 10 patients with radical nephrectomy and lymph node dissection for locally advance or metastatic RCC
Number of Sites	Single site: Indiana University Simon Cancer Center, Indiana University Hospital

Outcomes:	**Partial nephrectomy** PRIMARY • Assess the presence and extent of fluorescence of cT1 RCC during partial nephrectomy SECONDARY • Pathologic agreement of fluorescence and FR α by immunohistochemistry • Agreement between visual and fluorescent images • Inter-observer agreement of laparoscopic fluorescent images (for blinded surgeons) • Surgeon opinion of usefulness of intra-operative fluorescence imaging-Likert scale questionnaire (**Appendix A**) • Safety (perioperative, 10 days, 1-month)- Vital signs, hematology, chemistry, and adverse events (NCI-CTCAE v4.0)
	Radical nephrectomy and lymph node dissection PRIMARY • Assess the presence and extent of fluorescence in regional lymph nodes or metastasis in patients with RCC SECONDARY • Pattern of nodal spread • Agreement of nodal and primary tumor fluorescence • Pathologic agreement of fluorescence and FR α by immunohistochemistry • Detection of RCC in primary tumor in situ • Agreement between visual and fluorescent images. • Inter-observer agreement of in-situ fluorescent images (for blinded surgeons) • Surgeon opinion of usefulness of intra-operative fluorescence imaging- Likert scale questionnaire (**Appendix A**) • Safety (perioperative, 10 days, 1-month)-Vital signs, hematology, chemistry, and adverse events (NCI-CTCAE v4.0)

LIST OF ABBREVIATIONS AND TERMS

Abbreviation/Term	Definition
ADE	Adverse device effect
AE	Adverse event
BMP	Basic metabolic panel
CBC	Complete blood count
CMP	Comprehensive metabolic panel
CRF	Case report form
CT	Computed tomography
CTMS	Clinical Trial Management System
CTO	Clinical Trials Office
CTSI	Indiana Clinical and Translational Sciences Institute
DSMC	Data Safety Monitoring System
ECOG	Eastern Cooperative Oncology Group
FDA	Federal Drug Administration
FR	Folate receptor protein
FITC	Fluorescein isothiocyanate
GFR	Glomerular filtration rate
IDS	Investigational Drug Service
IHC	Immunohistochemistry
IND	Investigational new drug
IUH	Indiana University Hospital
IUSCC	Indiana University Simon Cancer Center
LND	Lymph node dissection
REDCap	Research Electronic Data Capture
RCC	Renal cell carcinoma
SAE	Serious adverse event
SEER	Surveillance epidemiology and end results

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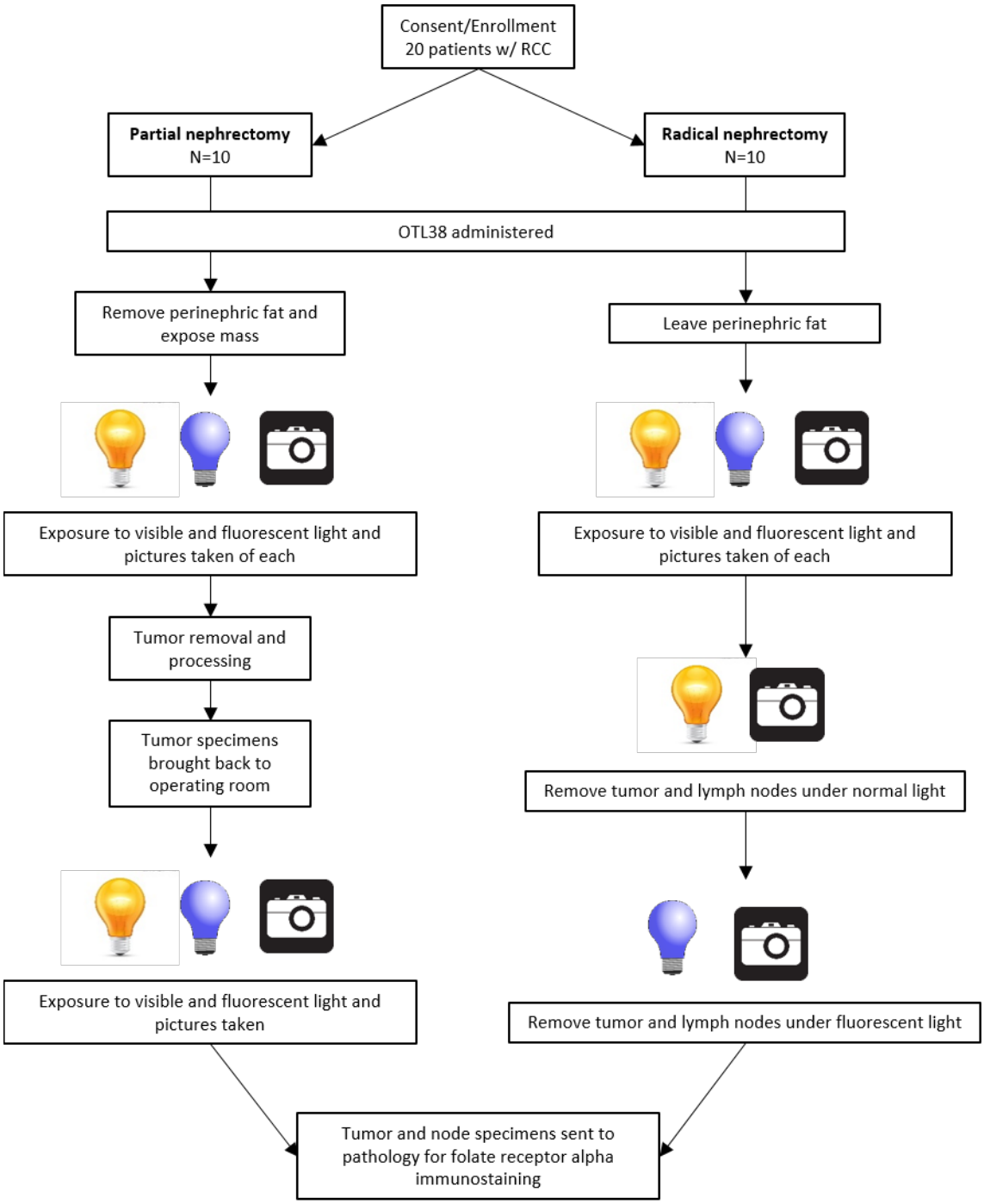
2 SCHEMA



3 BACKGROUND

Renal cell carcinoma (RCC) has the highest early mortality or potential years of life lost of all urologic malignancies and this trend is increasing according to the recent SEER database analysis from 1973 to 2006 (Kamel 2012). Computerized tomography (CT) imaging are used more often for detecting renal masses and has led to distinct groups of RCCs: localized (stage I-II) with a high cure rate and advanced (stage III-IV) with high rates of distant metastasis and early mortality. Partial nephrectomy and radical nephrectomy are standard treatments for renal

masses. Although these options demonstrate better survival, there are uncertainties related to the comparative effectiveness of these treatment options (Tan 2012). Nonetheless, surgery remains an integral part of the treatment strategy for all stages of RCC, and the ability to accurately image the disease in real time is becoming increasingly important for both localized and advanced disease.

3.1 Localized RCC

Localized RCC is a cancer of the kidney that has not spread to the lymph nodes or distant organs. Improved sensitivity of CT imaging procedure has led to the increased incidence of incidentally diagnosed, small, low stage RCC lesions than based on the classic triad of symptoms (hematuria, palpable mass, and pain) that occurs in less than 15% of RCC cases (Palapattu 2002). According to a study using the data form the National Cancer Database, a greater proportion of newly diagnosed patients with RCC present with localized RCC (lower stages) than in earlier years. The number of patients with Stage 1 localized RCC increased from 55% in 1993 to 67% in 2004 Kane 2008).

Sparing renal parenchyma in addition to cancer control has traditionally been viewed as an important and an unmodifiable variable in surgical treatment, especially in organ-sparing surgery or partial nephrectomy of localized RCC. Although healthy parenchymal safety margin during conservative renal surgery is still the standard of care, the risks of significant complications increase with the technical complexity of the case.

Limiting ischemia time has been used as an important modifiable factor during partial nephrectomy. Recent studies show that preoperative glomerular filtration rate (GFR) and percentage of parenchyma spared are the best predictors of long term GFR (Lane 2011; Simmons 2011). At the same time, the size of the safety margin during partial nephrectomy has not been effective in predicting recurrence (Berdjis 2006). Tumor enucleation for RCC (resecting along the pseudocapsule of the tumor instead of obtaining a safety margin) has been tried to spare renal parenchyma, limit blood loss, and resect anatomically challenging tumors. Contrasting results have been reported in studies relating the effectiveness of enucleation versus partial nephrectomy (Minervini 2009; Minervini 2011). Acceptance of enucleation has been slow due to the rate of pseudocapsule invasion and concern over incomplete tumor removal.

Minimally invasive treatment options for localized RCC include both standard laparoscopy and robot-assisted laparoscopic approaches. Robotic partial nephrectomy made up only 11.5% of all partial nephrectomies in 2008 (Yu 2012), but increased to nearly 40% by 2011 (Data not published from Nationwide inpatient sample). Therefore, we propose meeting this challenge by combining robotic surgery with novel means of targeted real-time imaging of RCC —folate tagged fluorescence —to confidently spare renal parenchyma while achieving 100% tumor removal. We propose evaluating folate-targeted fluorescence of the renal mass to see if it can be used as a real-time guide to surgeons during partial nephrectomies.

3.2 Advanced RCC

Between 1990 and 2006 the death rate decreased 21% on average for all malignancies in the US, but for kidney cancer it decreased by 6.9% (Jemal 2010). This placed kidney cancer 11th out of the 16 reported malignancies in the SEER database in terms of improving death rate. Despite the introduction of several new systemic therapies for advanced RCC, there are limited options that offer a reliable complete response. Cytoreductive nephrectomy continues to be the standard of

care, but the role for lymph node dissection is under investigation. We propose investigating the ability of real-time targeted imaging with folate tagged fluorescence to determine who should undergo lymph node dissection (LND) and the extent of that dissection.

Of those undergoing nephrectomy for localized disease, up to 18% will have recurrence or progression (Crispen 2008). Theoretically, many of these could benefit from a LND. However, as partial nephrectomy replaces radical nephrectomy for larger tumors, arguably fewer LNDs will be performed. Although previous studies have concluded that a LND can provide a durable progression free survival, the criteria for selection of patients who will most likely benefit from a LND remains a challenge (Delacroix 2011).

Templates for LNDs continue to vary based on surgeon preferences, however, a recent study examined the predictability of 5 previously identified high-risk pathologic features on lymph node metastases prior to developing surgical template for LNDs (Crispen 2008). Patients with 2 or more of the 5 criteria previously identified by Blute et al (Blute 2004) — nuclear grade 3 or 4, presence of a sarcomatoid component, tumor size ≥10 cm, tumor stage pT3 or pT4, and histological tumor necrosis— were considered high-risk for nodal metastases. A total of 64/169 (38%) patients had positive lymph nodes. The lymph node positivity rate was directly proportional with the number of criteria identified: 20%, 39%, 49%, and 50% for 2, 3, 4, and 5 criteria present, respectively. Lymph node spread was assessed by examining 5 different landing zones (right hilar, pre-caval, inter-aortocaval, pre-aortic, and left hilar). For right-sided tumors, 45% of positive cases had a lack of hilar region involvement. So, if only the hilar nodes (those closest to the kidney) were sampled, then 45% of positive node cases on the right would have been missed. The hilar nodes are the easiest to remove surgically during an LND and are often the only nodes removed. Intraoperative imaging of tumors can enable surgeons to visualize and perform LNDs that could result in improved clinical outcomes.

3.3 Rationale

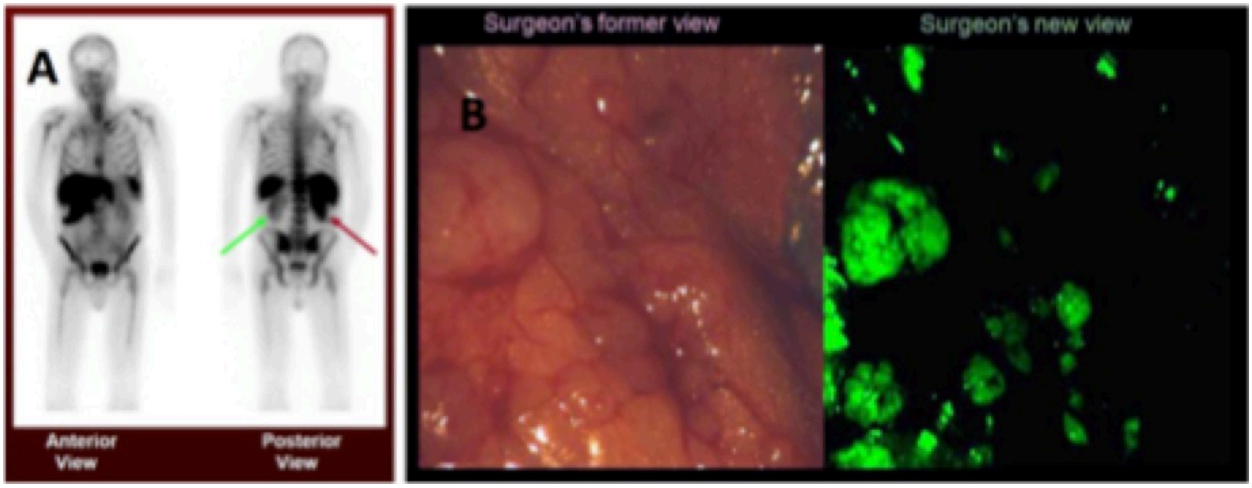
In 1989 Phil Low and graduate researchers at Purdue University began looking at the possibility of vitamins to act as a "Trojan horse" model and deliver targeted agents to cancer cells. His lab has continued this quest with one of the most promising agents being the vitamin folate. As Sega and Low et al note, folate fulfills many of the ideal characteristics for targeted agents: the folate receptor protein (FR) is upregulated in many cancer cells, while having limited expression on normal tissues, folate has a high affinity for the FR meaning saturation at low doses, folate conjugates are taken into the cancer cells by endocytosis, folate has a rapid clearance from the body cutting down nonspecific background noise during imaging ($t_{1/2}$ <10 min), folate's small size allows complete penetration of solid cancers, and folate's chemistry allows for attachment of nearly any therapeutic or imaging agent for delivery to cancer cells (Sega 2008). In one of its first applications, platinum resistant ovarian cancer, folate is being used to deliver therapeutic agents. It is the only treatment to improve progression free survival (22 vs. 12 weeks) in this population when compared to standard treatment (Naumann 2011).

While first purified as a tumor marker for ovarian cancer, Dr. Low has shown FR to be upregulated in numerous epithelial malignancies. Parker and Low et al demonstrated that RCCs are the second highest FR expressing cancer with 86% falling into the high positive category using a radioligand method (Parker 2005). There was some variability in expression within RCCs. Normal kidney proximal convoluted tubule cells also contained the FR on the apical and basal membranes, which is thought to either conserve or secrete plasma folate depending on the

needs of the body (Morshed 1997). Therefore, normal kidney cells are not expected to accumulate folate like cancer cells (0A).

A conjugate of Dr. Low's lab, folate and 99mTc, was recently used for SPECT imaging in 154 patients with proven or suspected cancer, of which 118 were RCC. In the RCC group 74% had mild or marked uptake of tracer in one or more lesions. For all cancer types, when comparing tracer uptake with immunohistochemistry (IHC) of previous or subsequent surgical specimens there was a 61% overall agreement. This was thought to be due to the IHC being specific for only one isoform of the FR or heterogeneity of FR expression between cancer lesions in the same patient (Fisher 2008). The agent was found to be safe and well tolerated.

Figure 1. Representative SPECT image and Immunohistochemical Staining of RCC



Dr. Low's lab recently developed OTL38, which is a folate analog conjugated with a fluorescent dye that emits light in the near infrared spectrum (near 800nm). This longer wavelength allows for deeper penetration of the fluorescent light through tissues with the potential to better image tumors beneath adipose tissue or deeper into organ parenchyma. A phase 1 trial was recently completed showing only mild adverse events at drug concentrations appropriate for further phase 2 trials.

Renal cell carcinoma is the only urologic malignancy that is increasing in both incidence and early mortality. Surgery is an integral part of the treatment strategy for all stages of RCC, and the ability to accurately image the disease in real time is becoming increasingly important for both localized and metastatic disease.

Folate targeted imaging was recently developed due to many compelling characteristics: the folate receptor **α** is up regulated in up to 75% of RCCs, incorporates the folate conjugate into the cancer cell by endocytosis, and has limited expression on normal cells. Multiple folate conjugates are being tested for staging, intraoperative imaging, and drug delivery and are showing unmatched survival benefits in platinum resistant ovarian cancer.

For localized RCC (stage I-II), the limits of renal sparing surgery are being explored to preserve healthy nephrons. Studies show that the percentage of parenchyma spared during partial nephrectomy is the best predictor of postoperative renal function. Folate targeted fluorescent imaging offers the ability to see tumor at the resection margin. The hypothesis is that folate imaging will correlate with final pathology at the border of the tumor and therefore be a

dependable guide for robotic resection. Additional hypotheses are that there will be strong agreement between surgeons on what has positive fluorescence, surgeons will find the folate imaging useful during resection and the procedure will be safe.

For advanced RCC (stage III-IV), folate imaging offers a better chance of complete resection than other treatment options currently available. While level-1 evidence within the literature shows LND in stage I-II RCC has no therapeutic benefit, its role in Stage III-IV disease may show some benefit (Blom 2009). Some groups advocate risk-stratifying of RCC using intraoperative frozen section analysis, which adds considerable cost and time to the surgery.

Intraoperative imaging of FR could improve selection for LNDs and provide real time guidance for the surgical template used. Overall, this will be the first study to use folate targeted intraoperative imaging of RCC to improve clinical outcomes. Data obtained from the proposed pilot study will help develop future protocols with large sample sizes and accurately estimate sensitivity, specificity, positive predictive value, and negative predictive value of OTL38.

4 STUDY OBJECTIVES

Primary

- To explore the use of OTL38 and fluorescence imaging to detect RCC in partial nephrectomy at the margins of resection, and in lymph node(s) or other metastases during radical nephrectomy.

Secondary

To assess the specificity of tumor fluorescence obtained using OTL38 with FRα+ renal cell carcinoma cancer lesions detected via immunohistochemistry

- To assess the agreement between fluorescence imaging using OTL28 and FRα by immunohistochemistry
- To assess the agreement between fluorescence imaging using OTL38 and visual/tactile assessments
- To assess the pattern of (lymph) nodal spread during radical nephrectomy
- To assess the degree of agreement of (lymph) nodal and primary tumor fluorescence during radical nephrectomy
- To assess the detection of RCC in primary tumor in situ during radical nephrectomy
- To assess inter-observer variations of tumor fluorescence images captured during surgery
- To assess physician rated usefulness of intra-operative fluorescence imaging- Likert scale questionnaire (**Appendix A**)
- To assess the safety of OTL38 (perioperative, 1-month)- Vital signs, hematology, chemistry, and adverse events (NCI-CTCAE v4.0)

5 RISKS AND BENEFITS OF OTL38

5.1.1 Risks

The issues of possible concerns with the use of the OTL38 and the imaging system are:

- Presence of an imaging system in the operating room
- Phototoxicity or thermal damage from the light source
- Nonspecific localization of OTL38
- Failure of OTL38 to bind to receptors
- Fading of the chromophore (photobleaching)
- Inability to excite the dye in OTL38 or to record emission
- Adverse events suggestive of hypersensitivity

5.1.1.1 Imaging system

The presence of an imaging system in the operating room is not novel and should not create a problem with maintaining a sterile field. In this case, the camera system will be used for intra-operative imaging prior to surgical resection to record the localization of tumors, and post-resection to document the visualization of any residual tumor. As such, it need not be intrusive during the procedure. Standard operating room procedures to ensure a sterile milieu, including appropriate draping of the imaging system, will be employed.

There is a potential for phototoxicity or thermal damage from any light source. The degree of risk is related to the power of the light, the duration of exposure, and the temperature of the light source near the patient. The maximum temperature and irradiance for the imaging system are well within established safety limits. Controls will be in place to ensure that the exposure to the imaging system is limited to that is necessary to identify the tumor lesions and capture images.

Like all chromophores (fluorophores), excitation by appropriate wavelength of light will result in molecular activation resulting in a different wavelength of light being emitted. This is an active process that changes the excitability of the molecule potentially leading to photobleaching. Since white light contains all wavelengths of light, extended room light exposure could lead to bleaching. Exposure to the excitation light source will be limited during the procedure. According to van Dam et al. (2011), the initial images can be captured in a mean time of 10 minutes (4-36 minutes), and tumor-specific fluorescence can be detected for up to 8 hours. The risk that the camera system will not excite the OTL38 dye or record an image after emission is minimal.

5.1.1.2 OTL38

While OTL38 appears to specifically localize RCC, there is a possibility that some 5-10% of patients will have and will not benefit from use of this agent. The risk of exposure to OTL38 to these patients FR-alpha-negative tumors and to patients without cancer appears small.

There is no evidence to date to indicate failure of OTL38 to bind to FRα, so this remains a theoretical concern. Possible mechanisms would be competitive antagonism with another ligand (eg, folate) or a change in the molecule or receptor to hinder binding.

The rat and dog were selected as the rodent and non-rodent toxicology species for the OTL0038 toxicology program. While all studies showed that test article related green(ish) discolorations

may occur with OTL0038, which is an opaque, dark green liquid following formulation, these discolorations were not signs of irritation or associated with toxicological findings (see investigator brochure). In in-vivo safety toxicology and safety pharmacology studies, doses as high as 30.8 mg/kg in Sprague Dawley rats and 13.9 mg/kg in Beagle dogs (>500X the proposed human starting dose of 0.025 mg/kg), were well tolerated and resulted in no adverse effects.

A phase 1a study was conducted on 30 healthy controls with 23 receiving OTL38 and 7 acting as placebo by 3:1 randomization. A single dose IV administration of 0.025, 0.05, 0.1, or 0.2 mg/kg was given. No serious adverse events or death occurred. A total of 83 mild and moderate AEs were noted in the 23 patients receiving OTL38. With the exception of sleepiness (somnolence), all were suggestive of hypersensitivity reactions and no adverse events were cardio-pulmonary in origin. The most frequent adverse events (AEs) were nausea (11/23, 47.8%), pruritus (9/23, 39.1%), abdominal discomfort (8/23, 34.8%), somnolence (8/23, 34.8%), and dizziness (5/23, 21.7%). One treatment was stopped after a 15% infusion (treatment #5) and one had infusion held prior to completion. Initial drug infusion was 20 mL infused over 10 minutes. However, the protocol was changed to 220 mL over 60 minutes in response to early moderate AEs. After the infusion was changed to 220 mL over 60 minutes, only mild AEs were observed and no infusion was stopped or held up for those receiving 0.1mg/kg or less.

Intravenous infusion over 60 min of 0.025 and 0.05 mg/kg OTL38 in a total volume of 220 ml 5% dextrose (D5W) to healthy volunteers was associated with mild adverse events that did not require any intervention, were not considered to be clinically significant, and there appeared to be no relationship to an increase in dose.

5.1.2 Benefits

The potential benefits of OTL38 imaging are:

- Improved staging of the tumor
- Removal of more lesions
- Added assurance of clean margins to excised tumors.

In a preliminary study of ovarian cancer by van Dam et al (Van Dam 2011), significantly more lesions were detected by surgeons using Folate-FITC than with visual observation alone (34 vs. 7; $p < 0.001$). Lesions that were as small as 1 mm could be detected and removed. This resulted in better staging of patients as more lesions could be visualized but, more importantly, it resulted in greater tumor debulking. Since the degree of tumor removal directly affects the prognosis, this was a large potential benefit. This would need to be studied with OTL38. Use of OTL38 would not change the surgeon's option to remove additional tissue because of impressions based on experience, visualization, or tactile senses.

6 ELIGIBILITY CRITERIA

6.1 Inclusion criteria for localized RCC treated with partial nephrectomy

To be considered eligible to participate in this study, a patient must meet all the inclusion criteria listed below:

- ≥ 18 years of age.
- Have primary or suspected diagnosis of RCC, with presence of cT1-2 renal mass by diagnostic CT assessment.
- Scheduled for partial nephrectomy of renal mass.
- Expected survival of at least 3 months.
- Written informed consent available.
- ECOG ≤ 1 (**Appendix G**).
- Negative serum or urine pregnancy test within 24 hours for females of child bearing age
- Recovered from toxicity of any prior therapy to ≥ grade 1.

6.2 Inclusion criteria for advanced RCC treated with radical nephrectomy

To be considered eligible to participate in this study, a patient must meet all the inclusion criteria listed below:

- ≥ 18 years of age.
- Have pathologic or suspected diagnosis of RCC with presence of cT1-4 renal mass and evidence of nodal or metastatic involvement by diagnostic CT assessment
- Scheduled for radical nephrectomy and lymph node dissection.
- Expected survival of at least 3 months.
- ECOG ≤ 2.
- Negative serum or urine pregnancy test within 24 hours for females of child bearing age.
- Recovered from toxicity of any prior therapy to ≥ grade 1
- Written informed consent available.

6.3 Exclusion criteria for both localized and advanced RCC

- History of any anaphylactic reaction, any severe allergy, or any allergy to folate.
- Brain metastases
- Baseline GFR < 50 mL/min/1.73m^2)
- Hepatic toxicity ≥ Grade 2 (using CTCAE version 4 standard definitions).
- Participation in another investigational drug trial either concurrently or 30 days prior to surgery
- Any medical condition that in the opinion of the investigators could potentially jeopardize the safety of the patient, limit the patient's ability to complete the study, and/or compromise the objectives of the study.
- Known sensitivity to fluorescent light

7 PATIENT REGISTRATION

Patient enrollment will take place over 24 months at a single institution-Indiana University Simon Cancer Center (IUSCC)/Indiana University Hospital (IUH).

All patients will be registered with the Indiana University (IU) Department of Urology. Regulatory files will be maintained by the Department of Urology. Applicable regulatory documents must be completed and on file prior to registration of any patients. Potential patients will be identified in the Urology clinic or by physician referrals. Patients who appear to be eligible for this trial will undergo the Informed Consent Process and be screened for eligibility utilizing the Eligibility Criteria. Individual patient registration will be done in the OnCore® database. The original signed Institutional Review Board (IRB) approved Informed Consent Document and completed eligibility checklist will be stored in the following location:

Indiana University Medical Center
Indiana Cancer Pavilion
Urology Clinic, 3rd floor
535 N. Barnhill Dr.
Indianapolis, IN 46202

8 STUDY PROCEDURES

8.1 Summary

Administration of the study drug will begin prior to skin incision in the preoperative area where safety monitoring will occur. The tumor resection occurs approximately 2 hours after being brought back to the operating room and so there is no wait time between infusion and being taken back to operating room. Intraoperative fluorescent imaging will be utilized in parallel with the standard operating procedure to capture images during surgery. Images also will be taken of the excised specimen on the back table. The excised specimen will be sent to the pathology department for fluorescent imaging and immunohistochemistry for FR. Subjects will have a 2-5 day hospital stay (normal nephrectomy recovery period) where safety measurements will be taken. Final safety measurements will be taken at the 10-day and 1-month follow-up visits.

8.2 Study calendar

Study Procedures[12]	Screening	Pre-surgery	Surgery	Post-surgery	Hospital Admission	Hospital Discharge	Follow-up Phone Call	Follow-up Appt
	Up to 90 days prior to surgery	Day 0			Days 1-5	Days 2-6	Day 10 (± 3 days)	1 Month (±14 days)
Informed consent	X							
Inclusion/exclusion criteria	X							
Demographics	X							
Medical history[1]	X	X[2]						
Physical exam	X							X
Height/weight	X							X[3]
Vital signs[4]	X	X[5]	X[6]	X[6]	X[6]	X[6]		X
Urine/serum pregnancy test	X[7]	X[8]						
Complete metabolic panel (CMP)								X
Basic metabolic panel (BMP)[9]	X				X[10]			
CBC w/ diff	X				X[10]			X
Study drug administration[11]		X						
Surgery with associated procedures including intra-operative imaging			X					
Tumor ex-vivo sample collection			X					
Pathologic assessment				X				
PI Likert questionnaire				X				
AE assessments		X	X	X	X	X	X	X
ADE assessments			X	X	X	X	X	X
Concomitant medications	X	X[1]	X	X	X	X	X	X

Footnotes:
[1]Includes past medical history, past surgical history, allergies and any ongoing medical conditions

[2]Since screening visit and up to 30 days before surgery
[3]Weight only
[4]Temperature, blood pressure, respiratory rate and pulse rate
[5]Taken at baseline (up to 30 minutes prior to OTL38 infusion), approximately every 15 minutes during infusion (only blood pressure and pulse rate), and then approximately every hour after infusion until surgery is initiated
[6]Record per institutional practice
[7]Serum pregnancy test for all females of childbearing potential
[8]Urine pregnancy test for all females of childbearing potential
[9]Basic metabolic panel includes Na, K, Cl, Mg, Ca, BUN, creatinine
[10]Obtain on day 1 after surgery
[11]Single dose of anti-histamine (diphenhydramine 25 mg IV) will be administered prior to the study drug; study medication will be administered less than 2 hours prior to the skin incision
[12]Patients from both arms will follow this schedule

8.3 Assessments by visit

8.3.1 Screening (Day -90 to Day -1):

- Informed consent: investigators or their designees will discuss with subjects the nature of the study, its requirements, risks, and restrictions to obtain informed consent for participation in the study. Subjects should have sufficient time to review the study information and consent form and to ask any questions necessary to make an informed decision regarding their participation in the study. Written informed consent is to be obtained before any other study-specific procedure.
- Eligibility criteria: make sure patient meets study eligibility criteria.
- Demographics
- Medical history: includes past medical history, past surgical history, allergies and any ongoing medical conditions
- Medication history: all medications noted in patients record at time of screening
- Physical examination: including height, weight, vital signs (blood pressures, heart rate, respiratory rate, and temperature). The extent of the physical examination is determined by the investigator (or designee) and is based on clinical relevance to the subject's medical history and scheduled surgical procedure as per standard medical/surgical care.
- Blood samples: complete blood count with differential (CBC w/ diff) and basic metabolic panel (Na, K, Cl, Mg, Ca, BUN, creatinine), and serum pregnancy for women of childbearing age

8.3.2 Surgery (Day 0):

8.3.2.1 Pre-surgery:

- Eligibility criteria: ensure subject continues to meet study eligibility
- Medical history: capture additional medical history since screening
- Concomitant medications: record medications taken since screening through day of surgery. All preoperative medications (this does not include normal saline or other routine maintenance intravenous fluids) will also be recorded.
- Urine pregnancy test: for women of childbearing age
- Vital signs (blood pressures, heart rate, respiratory rate, and temperature): will be taken at baseline (up to 30 minutes prior to infusion), approximately every 15 minutes during infusion (blood pressure and pulse rate), and then approximately every hour after infusion until surgery is initiated. During and post-surgery these will be recorded per institutional practice.
- Study drug administration:
    - A single dose of anti-histamine (diphenhydramine 25 mg IV) will be administered prior to the study drug.
    - Begin administration of study medication less than 2 hours prior to the skin incision on day of surgery in the preoperative area.
    - Record time of study medication administration
    - Record time of scheduled surgery (listed operating room time)
- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) noted after the start of the study medication that was not present at screening.

8.3.2.2 Surgery:

The da Vinci® Fluorescence Imaging Vision System, SPY Elite Imaging System or Visionsense VSiii 3DHD system will be used to identify tumor lesions under normal/visible light and fluorescent light prior to surgical excision of lesions, and again after surgical excision of lesions to identify persistent lesions. The da Vinci system or Visionsense VSiii system will be used for all partial nephrectomies (robotic) and for robotic radical nephrectomies. The SPY Elite will be used for open radical nephrectomies. See **Appendix D** and **Appendix E** for more detailed information about the da Vinci and SPY camera/imaging systems. Information about the Visionsense VSiii system can be found at http://www.visionsense.com/vsiii-system/.

The Investigator will record the start and stop time for each exposure to fluorescence (pre-resection, post-initial resection, other [give reason]) in the eCRF. The total time of fluorescence will be calculated automatically.

Status	Time On Fluorescent Light	Time Off Fluorescent Light
Before surgical excision		
After initial surgical excision		
Other (explain reason)		

The number and location of suspect tumor lesions detected under **normal light and/or by palpation** will be recorded on the schematic (**Appendix B**) using an "O". The number and location of suspect tumor lesions detected using **fluorescent light** will be recorded on the schematic using an "x" (for those lesions that were also identified under normal light and/or by palpation, the "x" will be superimposed on the "O" as "⊗"; and for those identified only under fluorescence, they will be recorded only by the "x").

Light source	Notation
Visible light	O
Fluorescent	X
Both	⊗

For Partial Nephrectomies

Surrounding perinephric fat will be removed as indicated for the renal mass. Then the renal mass will be visualized and photographed (video and still images) using visible light and fluorescent light. As described above, start and stop times of exposure to fluorescent light will be noted, and the number and location of fluorescent lesions related to the mass and surrounding renal parenchyma detected will be recorded on the schematic. Findings under visible light will also be recorded.

After the renal mass is removed, it will be processed by the operating surgeon and the frozen section pathology staff. The tumor margin will be inked and the tumor sectioned in 5mm thick cross-sectional slices. The tumor will then be returned to the operating room to be viewed and photographed with visible and fluorescent light. It will then be sent for pathologic processing as discussed in **Appendix B**.

For Radical Nephrectomies

The regional lympadenectomy will be performed as per the standard of care for the patient's renal mass. The renal mass will be visualized and photographed (video and still images) using visible and fluorescent light *through the surrounding perinephric fat*. The start and stop times of exposure to fluorescent light will be noted, and the number and location of fluorescent lesion/s related to the mass and surrounding renal parenchyma detected under fluorescent and visible light sources will be recorded on the schematic.

All identified nodal tissue from each marking ("O", "X", and "⊗") will be excised, as determined by the Investigator, *under normal light.* Following the initial excision under normal light, the field will be illuminated with *fluorescent light* again to detect any remaining fluorescence-positive lesions. Any such lesions will be noted with a "P" on a schematic sheet (**Appendix C**) and excised as determined by the Investigator. A unique identifier will be assigned to each excised lesion, and the location for each lesion will be recorded on the schematic and in the eCRF. The reason for not excising any remaining lesion(s) will be noted. If no additional lesions are observed, this will be noted on the schematic and in the eCRF.

The nodal tissue will be processed by the operating surgeon and the frozen section pathology staff. The lymph nodal tissue will be marked with suture or clips to help with anatomic orientation of the specimen, and then sent for pathologic processing as discussed in the study pathology manual.

To further assess agreement of gross fluorescence with pathology**,** at least one representative lymph node biopsy done. Once the specimen is removed, it will be processed by the surgical team on the back table and sent to pathology for microscopic examination and immunohistochemistry assessment to determine if it contains FRα+ cancer cells. The pathologist will be blinded to the fluorescence results of the tumor.

For All Surgeries

- Vital signs: recorded per institutional practice
- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.
- Adverse device effects (ADE) assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence. Changes could be present but are likely due to the temperature of the cauterization as lesions are excised.
- Concomitant medications: record all intraoperative medications (this does not include normal saline or other routine maintenance intravenous fluids)

8.3.2.3 Post-surgery:

- Post-surgery questionnaire: the surgeon will complete a questionnaire describing the usefulness of the fluorescence imaging for tumor removal and to achieve a negative margin (**Appendix A**).
- Blinded surgeon assessment: A co-investigator, blinded surgeon who was not involved with the case will view the photographs and complete a questionnaire to assess inter-observer agreement (**Appendix A**).
- Type of RCC excision (radical, partial), need for blood transfusion and estimated blood loss
- Vital signs: recorded per institutional practice

- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.
- ADE assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence.
- Concomitant medications: record all postoperative medications (this does not include normal saline or other routine maintenance intravenous fluids)

8.3.3 In-hospital admission (Days 1-5):

- Blood samples: complete blood count w/ differential (CBC w/ diff) and basic metabolic panel (BMP) *on day 1 after surgery*
- Vital signs: recorded per institutional practice
- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.
- ADE assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence.
- Concomitant medications: record all postoperative medications (this does not include normal saline or other routine maintenance intravenous fluids)

8.3.4 Hospital discharge (Day 2-6):

- Vital signs: recorded per institutional practice
- Review records relating to discharge: date and time that discharge order was written (must occur the same day)
- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.
- ADE assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence.
- Concomitant medications: record all postoperative medications (this does not include normal saline or other routine maintenance intravenous fluids)

8.3.5 Follow-up phone call (Day 10 [±3 days]):

- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.

- ADE assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence.
- Concomitant medications: record any new medications taken since discharge
- A letter will be sent to any subject not contacted after 3 attempts by telephone

8.3.6 Follow-up appointment (1 Month [± 14 days]):

- Physical examination: including weight and vital signs (blood pressures, heart rate, respiratory rate, and temperature). The extent of the physical examination is determined by the investigator (or designee) and is based on clinical relevance to the subject's medical history and scheduled surgical procedure as per standard medical/surgical care.
- Blood samples: complete blood count with differential (CBC w/ diff) and complete metabolic panel (CMP)
- AE assessment: including hypersensitivity reactions, which could include skin reactions, difficulty breathing, eye or nasal reactions, or gastrointestinal complaints, redness, tenderness, pain, or rash at the injection site or any clinically relevant abnormality (with exception of surgical scars) that was not present at screening.
- ADE assessment: the surgeon will examine the subject to confirm that there are no visible changes to skin (erythema or pigment darkening), tissue or organs related to fluorescence.
- Concomitant medications: record any new medications since discharge or follow-up phone call

9 TOXICITIES TO BE MONITORED

The safety measures included in this study are standard for studies of investigational drugs. Additional monitoring following administration of study drug is due to screening for a hypersensitivity reaction after administration of OTL38 and for any adverse events that might be related to fluorescent imaging.

Any clinically relevant abnormality (with exception of surgical scars) noted after the start of the study medication that was not present at the screening should be recorded as an adverse event (AE).

On Target, LLC will be notified of any serious adverse events (SAEs) that occur and are felt to be due to the investigational drug by the investigators.

More details about AEs and SAEs can be found in Section 15.

10 STUDY WITHDRAWAL/DISCONTINUATION

Subjects must be discontinued from the study for the following reasons:

- Pregnancy
- Withdrawal of consent

- Investigator deems withdrawal necessary at any time if it is determined that it is not in the subjects best interest to continue or if the subject is found to be noncompliant with study procedures.

If subject discontinues after administration of study drug, he or she will be encouraged to continue on study for safety procedures per protocol. Reason(s) for discontinuing must be clearly documented in the appropriate source documents.

11 DRUG INFORMATION

11.1 Identification and description of test article

OTL38 will be supplied in vials containing 3.0 mL of solution of 2 mg/mL OTL38 for a total of 6 mg of drug per vial.

Study medication should be stored in a freezer at temperature -20°C and with protection from light.

IU Health Investigational Drug Services (IDS) will prepare the IV solutions according to instructions provided by On Target Laboratories at the assigned dose. All solutions should be protected from light.

11.2 Packaging and labeling

The study medication will be packaged in vials and labelled by the Sponsor's clinical supplies designee.

The vial label will include:

Study:

Batch 20802.001Z

OTL38 (6mg in phosphate buffer saline 3ml

Store at -15°C to -25°C

For Clinical Trial Only, Keep out of reach of children

The outer package label will have the following information:

On Target Laboratories, LLC,
West Lafayette, IN 47906
Tel (765) 588-4547

Route of administration: injection

Quantity supplied: 6 mg OTL38 per vial

Pharmaceutical dosage form: Phosphate Buffered Saline (pH 7.4)

Storage conditions: keep frozen at temperature -20°C

CAUTION: New Drug – Limited by Federal Law to Investigational Use

Lot number: 20802.001Z

Manufacturing date: 08 October 2013

Retest date: October2015

11.3 Storage and handling of test article

Study medication should be stored in a freezer at -20°C temperature and with protection from light.

11.4 Study drug administration

OTL38 will be prepared following the Dose Preparation for OTL38 Injection Pharmacy Manual (see **Appendix I**). OTL38 will be administered intravenously over approximately 60 minutes, less than 2 hours before surgery. If the patient develops any symptoms or signs suggestive of a hypersensitivity reaction, the infusion may be interrupted and then resumed based on the clinical judgement of the investigator on the condition of the patient.

Note that OTL38 should not be mixed with other medicinal products and should not be given simultaneously through the same IV line as another medicine.

11.5 Treatment compliance

All doses of study medication must be prescribed as a written order on each subjects' medical chart in order for IDS to dispense study medication. All doses of study medication are to be administered under the direction of the investigator or physician trained by the investigator.

11.6 Drug accountability

The pharmacist or designee will sign for receipt of study medications upon delivery to IDS. Study medications are to be dispensed only for those subjects formally entered into the study. The actual dosing time of study medication is to be properly documented in the hospital medication administrative record.

At the end of the study, these records will be used to account for all study medication. After drug accountability is reconciled, all unused study medication will be disposed of according to IDS Standard Operating Procedures.

12 STATISTICAL CONSIDERATIONS

Statistical analysis of this study will be the responsibility of the Biostatistics and Data Management Core at the Indiana University Melvin and Bren Simon Cancer Center (IUSCC). Parameter estimates and relevant summary statistics will be reported where appropriate. For continuous variables, summary statistics will include number of subjects, mean, median, standard deviation, minimum and maximum. Categorical endpoints will be summarized using number of subjects, frequency, and percentages. Missing data will not be imputed. Data analysis will be performed in SAS Version 9.4. Additional exploratory analyses of the data will be conducted as deemed appropriate. Changes from this analysis plan will not require an amendment to the protocol unless it changes a significant feature of the protocol.

The statistical analysis methods are outline below.

12.1 Study design

A Pilot, Phase 2, Non-randomized, Single Institution Study

12.2 Study population

The enrolled population comprises all patients who meet the eligibility criteria and are registered onto the study and who are treated on this protocol.

12.3 Sample size

The study consists of a total of 20 patients divided equally between two treatment arms that will be studied simultaneously:

- Arm 1: 10- partial nephrectomy for localized RCC (no lymph nodes)
- Arm 2: 10- radical nephrectomy and lymph node dissection for locally advance or metastatic RCC.

A total sample size of 20 was determined to balance the competing goals of assessing the primary and secondary while not exposing more patients to an investigational procedure than is required. Also, there is an 88% probability of seeing at least one toxicity event if the probability of that toxicity is 0.10. Thus, we feel confident that with a sample of size 20, we will be able to detect unacceptable toxicities that have probabilities of 0.10 or higher.

A sample size of 10 for each arm was chosen. If we do not observe sensitivity for detection of RCC of at least 70% in each arm, we will consider further refinement of the methodology before proceeding with additional larger studies.

12.4 Study endpoints

Partial nephrectomy

PRIMARY

- Detect the presence and extent of fluorescence of cT1 RCC in partial nephrectomy specimens at the margins of resection.

SECONDARY

- To assess the specificity of tumor fluorescence obtained using OTL38 with FRα+ renal cell carcinoma cancer lesions detected via immunohistochemistry
- To assess pathologic agreement between fluorescence imaging using OTL28 and FRα by immunohistochemistry
- To assess the agreement between fluorescence imaging using OTL38 and visual/tactile assessments
- To assess inter-observer variations of tumor fluorescence images captured during surgery
- To assess physician rated usefulness of intra-operative fluorescence imaging- Likert scale questionnaire (**Appendix A**)
- To assess the safety of OTL38 (perioperative, 1-month)- Vital signs, hematology, chemistry, and adverse events (NCI-CTCAE v4.0)

Radical nephrectomy and lymph node dissection

PRIMARY

- To detect the presence and extent of fluorescence in regional lymph nodes and metastases in patients with RCC.

SECONDARY

- To assess the pattern of (lymph) nodal spread during radical nephrectomy
- To assess the degree of agreement of (lymph) nodal and primary tumor fluorescence during radical nephrectomy
- To assess the detection of RCC in primary tumor in situ during radical nephrectomy
- To assess the pathologic agreement of fluorescence and FRα by immunohistochemistry
- To assess the agreement between fluorescence imaging using OTL38 and visual/tactile assessments
- To assess inter-observer variations of tumor fluorescence images captured during surgery
- To assess surgeon opinion of usefulness of intra-operative fluorescence imaging-Likert scale questionnaire (**Appendix A**)
- To assess the safety (perioperative, 1-month) of OTL38-Vital signs, hematology, chemistry, and adverse events (NCI-CTCAE v4.0).

12.5 Participant characteristics

Demographics, medical history, and physical examination characteristics will be summarized using descriptive statistics.

12.6 Concomitant medications

Concomitant medications will be coded using the WHO Drug dictionary and will be summarized in tabular format.

12.7 Analysis of primary objective

The primary endpoint is to see if OTL38 and fluorescence imaging has sensitivity to detect RCC in the primary tumor (for partial nephrectomy) or in lymph nodes (for radical nephrectomy with lymph node dissection). Both sensitivity and the secondary endpoint of specificity will be assessed as follows: Final pathology and immunohistochemistry will serve as the gold standard with which gross observation and fluorescent imaging are compared for margin of resection, regional lymph nodes, and primary tumor. Pathology and immunohistochemistry results will be listed per patient. Times on and off for the imaging system will be listed per patient. Sensitivity, specificity, positive predictive value, and negative predictive value will be calculated for descriptive purposes although we understand the sample sizes are not large enough to estimate these values with a high level of accuracy at this stage. That would be accomplished is a future larger study. Sensitivity and specificity will be estimated using the method of Generalized Estimating Equations (GEE), which will take into consideration the correlated nature of multiple lesions removed from the same patient. Each patient will have all excised target lesions contributing to the data analysis (each type: O, x,⊗, and P).

	Cancer +	Cancer-
Fluorescence+	True Positive (TP)	False Positive (FP)
Fluorescence-	False Negative (FN)	True Negative (TN)

Sensitivity (proportion of true positives) = (TP/TP+FN) x 100
Specificity (proportion of true negatives) = (TN/FP+TN) x 100

Sensitivity will be estimated using the GEE model for binomial distribution with fluorescence (-, +) as the fixed effect and patients as a random effect, and the 95% lower one-sided confidence interval for the true sensitivity will also be estimated. Specificity will be estimated using the GEE model for binomial distribution with fluorescence (-, +) as the fix effect and patients as a random effect, and the 95% lower one-sided confidence interval for the true specificity will also be estimated. Positive and negative predictive value will be estimated using similar methods.

12.8 Analysis of secondary objectives

Specificity: The analysis for specificity is described above.

Detection of RCC in primary tumor (radical nephrectomy arm only): This analyses will be the same as for the primary objective in the partial nephrectomy arm. Pattern of nodal spread will be described qualitatively.

Agreement between pathological agreement between fluorescence and FRα by IHC: This endpoint will also be assessed by Kappa statistics (for presence/absence) or Intra Class Correlation (for numerical measures). Agreement between nodal and primary tumor fluorescence in the radical nephrectomy arm will be assessed similarly.

Agreement between visual and fluorescent images: Kappa statistics will be used to assess agreement between visual and fluorescent images. In addition, the number of lesions detected by

usual visible/tactile conditions and/or palpation vs fluorescent imaging will be analyzed using a paired t-test.

Inter-observer agreement: Kappa statistics will be used to assess inter-observer agreement between of captured fluorescent images.

Usefulness: Responses to the Likert scale questionnaire completed by the surgeons will be listed and summarized

Safety: Vital signs will be summarized using descriptive statistics. Clinical chemistry, hematology, and urinalysis laboratory values will be listed in tabular format and those with above or below normal range will be identified within the table. AEs and TEAEs will be summarized by body system and presented by severity and causal relationship to study drug. ADEs will be summarized by body system and presented by severity and causal relationship to study device. Patients experiencing serious AEs or serious ADEs will be listed and summarized in tabular format.

13 DATA FORMS AND SUBMISSION SCHEDULE

Individual patient registration will be done in the OnCore® database. The OnCore® database is a comprehensive, web-based, Clinical Trial Management System (CTMS) which utilizes an Oracle database. OnCore® was developed by Forte Research Systems, Inc. and is used by the IUSCC Clinical Trials Office (CTO) and supported by the Indiana Clinical and Translational Sciences Institute (CTSI). OnCore® properly used is compliant with Title 21 CFR Part 11.

OnCore® provides users secure access with unique IDs/passwords and restricts access by assigned roles, from any location, to record, manage, and report on data associated with the operation and conduct of clinical trials.

All source documents are to remain in the patient's clinic file. All documents should be kept according to applicable federal guidelines. Clinical trial data in OnCore® are periodically monitored by the IUSCC Data Safety Monitoring Committee (DSMC).

REDCap Database

This study will utilize electronic Case Report Form completion via the secure, web-based, Research Electronic Data Capture (REDCap) system for data input. REDCap was developed by Vanderbilt University and is provided by Indiana University through their community license. REDCap is managed by the Indiana University Department of Biostatistics and secured by University Information Technology Services Advanced IT Core. Access to the password protected database will be limited to the investigators of this study, and any data that is distributed will be either de-identified or authorized by written permission from the subject.

14 PATIENT CONSENT AND PEER JUDGEMENT

The protocol and informed consent form for this study must be approved in writing by the appropriate Institutional Review Board (IRB) prior to any patient being registered on this study.

Changes to the protocol, as well as a change of principal investigator, must also be approved by the Board. Records of the IRB review and approval of all documents pertaining to this study

must be kept on file by the investigator and are subject to inspection at any time during the study. Periodic status reports must be submitted to the IRB at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination.

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

15 DATA AND SAFETY MONITORING PLAN

Investigators will conduct continuous review of data and patient safety. **Monthly review meetings** for moderate risk trials are required and will include the principal investigator, clinical research specialist and/or research nurse (other members per principal investigator's discretion). **Monthly** meeting summaries should include review of data, the number of patients, significant toxicities as described in the protocol, and responses observed. Summaries will be submitted and reviewed monthly by the DSMC (**Appendix H**). Submit to DSMC@iupui.edu.

15.1 Study Auditing and Monitoring

All trials conducted at the IUSCC are subject to auditing and/or monitoring. Reports will be reviewed by the full DSMC at the time of study review (Reference Risk Table in full DSMC Charter).

15.2 Early Study Closure

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the patient warrant early closure of the study, this recommendation should be made in writing to the DSMC. Alternatively, the DSMC may initiate suspension or early closure of the study based on its review of the investigator reports.

15.3 Reporting Guidelines

The DSMC has streamlined the reporting process by utilizing reports from OnCore®. This has allowed direct view of reports within the Clinical Trials Management System (CTMS); thus discontinuing paper reports. SAE reports are entered into OnCore® monthly and reviewed by the DSMC chair and/or coordinator monthly. Findings will be reported to the full DSMC at the time of study review.

15.4 Reporting Death

Death will be reported per local IRB reporting guidelines.

15.5 Study Accrual Oversight

Accrual data will be entered into OnCore® system. The Protocol Progress Committee (PPC) reviews study accrual twice per year while the PPC coordinator reviews accrual quarterly.

15.6 Protocol Deviations

Protocol deviations are entered into OnCore® and reviewed by the DSMC chair and/or coordinator monthly. Findings will be reported to the full DSMC at the time of study review.

15.7 Unanticipated Problems

Investigators are required to submit unanticipated problems to the DSMC concurrent with their submission of them to the IRB. **Prompt** reporting of unanticipated problems to the IRB is defined as within 5 days for on-site studies.

Unanticipated problems that will be reported **promptly** to the IRB include:

- Major protocol deviation/violation
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject (e.g. purposeful and for subject safety)
- Complaint of a subject that indicates unexpected risks, or complaint that cannot be resolved by the research team
- Publication in the literature, safety monitoring report, interim result or other finding that indicates an unexpected change to the risks or potential benefits of the research, in terms of severity or frequency
- Change in FDA labeling or withdrawal from marketing of a drug, device, or biologic used in a research study
- Investigator- or sponsor-initiated suspension or hold
- Serious or continuing non-compliance
- Adverse events (see section below)

16 ADVERSE EVENTS

16.1 Definitions of Adverse Events

16.1.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event can be any unfavorable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a drug, without any judgment about causality. Adverse events will be graded according to the NCI Common Toxicity Criteria, Version 4.0 (**Appendix F**).

16.1.2 Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence resulting in one or more of the following:

- Results in death or ANY death occurring within 28 days of last dose of study drug (even if it is not felt to be drug related)

- Is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization

  NOTE: Hospitalizations that are not considered SAEs are:

  - Hospitalization planned prior to first administration of study drug
  - Hospitalization for elective treatment of a pre-existing condition unrelated to the study medication
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.
- Pregnancy. Pregnancy of a patient or of the female partner of a male patient during the study or within 30 days after the last dose of study drug should be reported via an SAE report. Should pregnancy occur in a female participant during the treatment period, study drug should be discontinued and OnTarget notified immediately. Should a pregnancy occur in a female companion of a male participant during the treatment period, the male participant can continue treatment and OnTarget notified immediately. Any such pregnancy is to be followed until final outcome.

16.1.3 Unexpected Adverse Event

An adverse event not mentioned in the Investigator's Brochure or package insert or the specificity or severity of which is not consistent with the Investigator's brochure or package insert.

16.1.4 Determining Attribution to the Investigational Agent(s)

Attribution: An assessment of the relationship between the AE and the medical intervention. CTCAE does not define an AE as necessarily *"caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly **NO**T related
	Unlikely	The AE is **doubtfully related**
Related to investigational agent/intervention	Possible	The AE **may be related**
	Probable	The AE **is likely related**
	Definite	The AE **is clearly related**

16.2 Adverse Device Effects Definitions

16.2.1 Adverse Device Effect (ADE)

An ADE is defined as any untoward and unintended response to a medical device, in this study, the camera/imaging system. This definition includes any event resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device, as well as any event that is the result of a user error [ISO 14155-1:2003 (E) 3.1].

All ADEs noted during the study will be reported in the eCRF. Please refer to section 8.3.2.2 for a list of possible ADEs related to the camera/imaging system.

16.2.2 Suspected Adverse Device Effect

A suspected ADE is a subset of all ADEs for which there is a reasonable possibility (i.e., evidence to suggest a causal relationship between the device and the ADE) that the device caused the event. Suspected ADE implies a lesser degree of certainty about causality than adverse device effect.

16.2.3 Unanticipated Adverse Device Effect

An unanticipated ADE is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. [21 CFR 812.3 (s)]

16.3 Adverse Event Reporting Requirements:

Adverse events will be recorded from the time of first study drug administration and for at least 30 days after treatment discontinuation, regardless of whether or not the event(s) are considered related to trial medications. All AEs considered related to trial medication will be followed until resolution, return to baseline, or deemed clinically insignificant, even if this occurs post-trial.

16.3.1 Reporting to the FDA:

Per CFR 312.32 (c), the investigator-sponsor of the investigational new drug (IND) must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any adverse experience. There are two types of reports to the FDA: 7-day and 15-day reports.

16.3.1.1 15-Day IND Reports:

The investigator-sponsor of the IND must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any adverse experience:

- **associated with use of the drug** that is ***both***
- **serious *and***
- **unexpected**

Each written notification shall be made as soon as possible, and no later than **15 calendar** days after the investigator-sponsor's initial receipt of the information.

16.3.1.2 7-Day Reports:

The investigator-sponsor must notify FDA and all participating investigators in a written IND safety report of any adverse experience:

- **fatal or life-threatening experience** that is ***both***
- **associated with use of the drug *and***
- **unexpected**

The FDA will be notified as soon as possible but no later than **7 calendar** days after initial receipt of the information.

Report Content:

Each written notification may be submitted on FDA Form 3500A or in a narrative format and must bear prominent identification of its contents (i.e., "IND Safety Report"). For purposes of this protocol, the **MedWatch Report Form (FDA 3500A mandatory reporting), along with FDA Form 1571, and a cover letter** submitted to the appropriate FDA division, will serve as the written IND safety report. Follow-up information to a safety report should be submitted as soon as the relevant information is available.

Submit:

- MedWatch Report Form (FDA 3500A)
- FDA Form 1571
- Cover Letter

The IUSCC Protocol Development Coordinator should be contacted to assist with all FDA submissions and will be provided with a copy of all events that are reported to the FDA. All IND submissions will be maintained in a master file in the Clinical Research Office of the IU Simon Cancer Center.

16.3.2 Reporting to the IRB:

Unanticipated problems involving risks to subjects or others will be reported **promptly** to the IRB if they:

- unexpected;
- related or possibly related to participation in the research; and
- suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the serious adverse event does not meet all three (3) criteria listed above, the event does not have to be promptly reported to the IU IRB. However, it should be reported at the time of continuing review.

Prompt reporting of unanticipated problems to the IRB is defined as within 5 days from becoming aware of the event.

16.3.3 Reporting to the IUSCC Data Safety Monitoring Committee:

Regardless of study sponsorship, the study team must enter all initial and follow-up SAE, expedited, and noncompliance reports into OnCore® for review by the DSMC chair and/or coordinator. Expedited reports may include IRB Prompt Report Forms, AdEERS reports, MedWatch, and additional SAE forms as required by the sponsor. When follow-up information is received, a follow-up report should also be created in OnCore®. This DSMC reporting requirement is in addition to any other regulatory bodies to be notified (i.e. IRB, FDA, pharmaceutical company, etc.). The DSMC chair and/or coordinator will review all SAE, expedited, and noncompliance reports monthly.

16.3.4 Reporting to OnTarget, LLC:

On Target, LLC will be notified of any serious adverse events (SAEs) that occur and are felt to be due to the investigational drug by the investigators. On Target will be notified on the same schedule as IRB reporting (within 5 business days from becoming aware of the event).

Notify Timothy Biro, COO, On Target Laboratories at (216) 533-3082 and email: TBiro @ontargetlabs.com, and Ray Lamy, Regulatory Consultant, (480) 620-8897 and email: Arciel.llc@cox.net

17 PATIENT CONSENT AND PEER JUDGEMENT

The protocol and informed consent form for this study must be approved in writing by the appropriate IRB prior to any patient being registered on this study.

Changes to the protocol, as well as a change of principal investigator, must also be approved by the Board. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to inspection at any time during the study. Periodic status reports must be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination.

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

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19 APPENDICES

19.1 Appendix A

Assessments

19.1.1 Partial nephrectomy

Intraoperative surgeon assessment tool

Primary surgeon questionnaire: fill out immediately following completion of case.

1. What camera was used during the case?
    a. da Vinci® Fluorescence Imaging Vision System
    b. Visionsense VSiii 3DHD

2. At what time was the fluorescent camera first used during the case?
    a. _____

3. List all organs or structures with fluorescence:
    a. _____

4. Does the tumor show fluorescence?
    a. Very bright fluorescence
    b. Bright fluorescence
    c. Mild fluorescence
    d. Minimal fluorescence
    e. No fluorescence

5. Does the normal kidney surrounding the tumor show fluorescence?
    a. Very bright fluorescence
    b. Bright fluorescence
    c. Mild fluorescence
    d. Minimal fluorescence
    e. No fluorescence

6. Compare fluorescence between tumor and kidney:
    a. Tumor is a lot more than the kidney
    b. Tumor is more than the kidney
    c. Tumor is the same as the kidney
    d. Tumor is less than the kidney
    e. Tumor is a lot less than the kidney

7. Does fluorescence aid in locating the renal tumor?

    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

8. Does fluorescence help resect the tumor?

    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

9. Does fluorescence help to identify the border or margin of the tumor during resection?

    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

10. If commercially available would you use this fluorescent product for partial nephrectomies?

    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, most likely not
    e. No, for sure not

11. Based on white light examination after tumor removal, are there any visible changes to organs (erythema or pigment change)?

    a. Yes, explain_____
    b. No

12. Based on white light examination after tumor removal are there any visible changes to skin (erythema or pigment darkening)?

    a. Yes, explain_____
    b. No

Blinded surgeon assessment of intraoperative photos

To be completed by surgeons not involved in case

1. Photographs taken with and without fluorescent imaging just inside trocar showing hemi-abdomen: Gerotas, colon, and liver (right); colon, spleen, stomach (left).
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor.

2. Photographs with and without fluorescent imaging taken after Gerotas removed showing tumor and surrounding normal parenchyma.
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor.

3. Photographs with and without fluorescent imaging taken during resection of tumor.
    a. Place “X” over any fluorescing regions
    b. Place “O” on white light images over tumor areas

4. Photographs with and without fluorescent imaging taken in tumor fossa after tumor removal.
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor.

Ex-vivo assessment of removed tumor

Primary surgeon to complete on back table immediately following case. All images are in digital full color.

1. What camera was used for ex-vivo assessment?
    a. da Vinci® Fluorescence Imaging Vision System
    b. Visionsense VSiii 3DHD

2. Gross fluorescent images of specimen 90° from each other by rotating about central axis. Also, upper and lower pole images (6 faces of specimen).

3. Does the tumor show fluorescence?

    a. Very bright fluorescence
    b. Bright fluorescence
    c. Mild fluorescence
    d. Minimal fluorescence
    e. No fluorescence

4. 5mm slices through specimen with both fluorescent and white light image of each slice. Calibration measurement in photo.

5. At least 1 representative sample will be sent of primary tumor for FR- α assessment

19.1.2 Radical nephrectomy

Intraoperative surgeon assessment tool

Primary surgeon questionnaire: fill out immediately following completion of case.

1. What camera was used during the case?
    a. da Vinci® Fluorescence Imaging Vision System
    b. Visionsense VSiii 3DHD
    c. SPY Elite

2. At what time was the fluorescent camera first used during the case?
    a. _____

3. List all organs or structures with fluorescence:
    a. _____

4. Does the tumor show fluorescence?
    a. Very bright fluorescence
    b. Bright fluorescence
    c. Mild fluorescence
    d. Minimal fluorescence
    e. No fluorescence

5. Does fluorescence aid in locating the lymph nodes or metastasis?
    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

6. Does fluorescence help with this patient's radical nephrectomy?
    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

7. Does fluorescence help to identify the border or margin of the tumor during radical nephrectomy?
    a. Yes, a lot
    b. Yes, somewhat
    c. Not sure
    d. No, somewhat worse than without fluorescence
    e. No, a lot worse than without fluorescence

8. Based on white light examination after tumor removal, are there any visible changes to organs (erythema or pigment change)?
    a. Yes, explain_____
    b. No

9. Based on white light examination after tumor removal are there any visible changes to skin (erythema or pigment darkening)?
    a. Yes, explain_____
    b. No

Blinded surgeon assessment of intraoperative photos

To be completed by surgeons not involved in case

1. Photographs taken with and without fluorescent imaging just inside trocar showing hemi-abdomen: Gerotas, colon, and liver (right); colon, spleen, stomach (left).
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor or lymph node.

2. Photographs with and without fluorescent imaging taken after exposure has been obtained to the nodal areas or sites of metastasis
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor or lymph node.

3. Photographs with and without fluorescent imaging taken during resection of tumor.
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor or lymph node.

4. Photographs with and without fluorescent imaging taken in tumor fossa after tumor removal.
    a. Place “X” over any fluorescing regions and draw outline of each fluorescing area
    b. Place “O” on white light images over tumor areas and draw outline of suspected tumor or lymph node.

Ex-vivo assessment of removed nodes/metastasis

Primary surgeon to complete on back table immediately following case. All images are in digital full color.

1. What camera was used during ex-vivo assessment?
    a. da Vinci® Fluorescence Imaging Vision System
    b. Visionsense VSiii 3DHD
    c. SPY Elite

2. Gross fluorescent images of each enlarged lymph node or metastasis removed

3. Does the tumor show fluorescence?
    a. Very bright fluorescence
    b. Bright fluorescence
    c. Mild fluorescence
    d. Minimal fluorescence
    e. No fluorescence

4. 5mm slices through specimen (or bilvalve if < 2cm) with both fluorescent and white light image of each slice. Calibration measurement in photo.

5. At least 1 representative sample will be sent of primary tumor for FR- α assessment

19.2 Appendix B

Pathology Sample Procedures

Immunohistochemical procedures:

Tissue Processing: Tissues were fixed overnight at room temperature in either 10% NBF after which they were transferred through graded concentrations of alcohol to xylene inside a Leica Automated Vacuum Tissue Processor. They were embedded in paraffin before being sliced into five micron thick sections, mounted onto positively charged slides, baked at 60 C, and then were cut and stained for routine H&E.

Immunostaining:

The slides were then deparaffinized in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed by immersing the slides in Target Retrieval Solution (DAKO) for 20 min. @ 90 degrees C. (in a water bath), cooling at room temperature for 10 min., washing in water and then proceeding with immunostaining. All subsequent staining steps were performed on the Dako Immunostainer; incubations were done at room temperature and Tris buffered saline plus 0.05% Tween 20, pH 7.4 (TBS - Dako Corp.) was used for all washes and diluents. Thorough washing was performed after each incubation. Slides were blocked with protein blocking solution (Dako) for 45 min.; after washing, 5 µg/ml of the primary antibody, Folate Receptor alpha (Biocare) was added to the slides and incubated 30 min to 45 minutes at room temperature. Following washing in TBST, visual detection was performed with the Envision Flex Polymer link, and DAB chromogen (DAKO). The slides were washed, coverslipped and examined using brightfield microscopy. Control sections were treated with an isotype control using the same concentration as primary antibodies to verify the staining specificity.

Whole Slide Digital Imaging:

The Aperio whole slide digital imaging system was used for imaging. The Aperio Scan Scope CS system was used (360 Park Center Drive, Vista, CA 92081). The system imaged all slides at 20x. The scan time ranged from 1 ½ minutes to a maximum time of 2.25 minutes. The whole images were housed and stored in their Spectrum software system and images were shot from the whole slides.

Computer-assisted morphometric analysis of digital images will be done using the Aperio software that came with the Aperio Imaging system. (The positive pixel algorithm which is the only FDA approved algorithm for clinical trials).

Automatic Image Quantitation:

The Positive Pixel Count algorithm was used to quantify the amount of a specific stain present in a scanned slide image. The Positive Pixel Algorithm was altered with a hue value of 0.62, hue width of 0.40, and color saturation threshold of 0.005. A spectrum of color (range of hues and saturation) and intensity (weak, positive, and strong) were masked and evaluated. The algorithm counted the number and intensity-sum in each intensity range, along with three additional quantities: average intensity, ratio of strong/total number, and average intensity of weak positive pixels. Only staining classified as "positive" or "strong positive" was used to calculate positivity; regions classified as "weak positive" were mostly cytoplasmic and background staining, and were not counted. The algorithm had a set of default input parameters when first selected—these inputs have been pre-configured for Brown color quantification in the three intensity ranges (220-175, 175-100, and 100-0). Pixels which were stained, but did not fall into the positive-color specification, were considered negative stained pixels—these pixels were counted as well, so that the fraction of positive to total stained pixels could be determined. The ratio of the 'Positive' and 'Strong Positive' to the total number of pixels provided the positivity percentage for each sample. The algorithm was applied to an image using ImageScope. This program allows for the selection of an image Region of Analysis and the specification of input parameters. After this is completed, the algorithm can be applied and the results can be viewed.

19.3 Appendix C

Surgical Reporting Schematics

posterior

19.4 Appendix D

da Vinci® Fluorescence Imaging Vision System

Table of Contents

19.4.1 Prior Investigations with the Device

This is the first clinical trial in the United States, to employ the da Vinci® Fluorescence Imaging Vision System for the intraoperative visualization of renal cell carcinoma labeled with OTL38.

The critical operational features in a fluorescence camera system include a light source capable of providing consistent and even illumination to the surgical field, appropriate excitation and emission filters in the light path for OTL38 fluorescence, image collection hardware and software, and a system that does not impede the surgeon or compromise the sterility of the surgical field.

There are many potential designs for a camera system intended to visualize -labeled ligands like OTL38 in situ. The critical design features in terms of efficacy are:

- proper wavelength of excitation
- detection of proper wavelength of emission
- limiting the detection of autofluorescence or artifacts
- providing enough illumination to excite the molecule
- avoiding photobleaching
- appropriate size of area of illumination

The critical features in terms of safety are:

- avoiding phototoxicity to patient or staff
- limiting the risk of burns
- limiting exposure to potentially damaging illumination

The potential efficacy between an experimental system as was used in a study by van Dam et al (2011) or an FDA-cleared system is determined by critical design features. In addition, the commercial system, such as the da Vinci® Fluorescence Imaging Vision System, will provide greater consistency across sites. The OTL38 dye will be detected in the near infrared spectrum with the da Vinci® Fluorescence Imaging Vision System. Autofluorescence is less of a problem with a near infrared dye (such as incorporated in OTL38), than with a dye that fluoresces in the visible light range (such as fluorescein). Autofluorescence can be related to at least three factors. One is intracellular components that appear as bright objects, although rarely the same shade or brightness with OTL38. These appear to increase with aging in many species. A second factor is extraneous wavelength light, which can illuminate tissue and obscure fluorescein fluorescence - this is controlled for with barrier filters; use of a near infrared dye, such as the OTL38 dye, decreases the likelihood of extraneous wavelength light, given the OTL38 dye excites at wavelengths higher than the visible light range. A third factor is an edge or reflection effect, in which edges or variations in tissue become highlighted. This autofluorescence effect disappears as the angle of illumination is changed; the OTL38 dye fluorescence will not change.

The human eye is far superior to any camera system in capturing light and resolving an image. This is evident to anyone familiar with low-light photography where the camera must be held firmly for even a half-second to many seconds to capture what the eye resolves immediately. In fluorescence microscopy or photography, the amount of light is further diminished by the use of excitation and emission filters. Thus, the camera system may take considerably longer to record labeled tumors than does the surgeon. To enhance the capabilities of the camera system, a number of hardware and software integrations are made in the system, optimizing image capture and enhancement.

The deciding factors in choosing a camera system are related to safety, efficacy, and the manufacturer's ability to supply the appropriately tailored system. There are a number of variables, some biological, that affect efficacy. After an initial dose of intravenous OTL38, the compound will be widely dispersed in the body. Following a period of washout, only regions with a high folate receptor density are expected to retain the label. There is no predetermined critical saturation point that needs to be reached to make a tumor visible, and a lower concentration of labeling can be compensated for with a longer time of illumination and image capture. An excess of labeling can lead to decreased fluorescence through quenching, whereby neighboring emitted photons interfere with each other. This quenching would diminish over time as the concentration of label continued to decrease. The presence of labeling on a suspected tumor mass will not be the determining factor in whether the surgeon removes the mass or not in this study. The imaging is an adjunct to the surgeon's usual procedures of visualization, palpation, and experience.

A critical safety feature of the system is avoiding phototoxicity to the surgical area, surrounding tissues, or retinal tissues in patient or staff. Tissue damage can result either from exposure to high-intensity light or to heat generated by a light source. Injury to the surgical area is unlikely as the light is restricted by a barrier filter and thus contains only a fraction of the light energy released from the source. Both the light energy and the heat produced by this system are unlikely to cause tissue damage (**Appendix D & Appendix E**). A laser is present in the light source, and the IEC 60825-1:2007 standard for laser safety is applied to the light engine. This standard provides exposure limits that apply to any light source. For skin, in the wavelength region from 700-800 nm, the MPE (maximal permissible exposure) for skin tissue is set at 200 mW/cm^2. The duration for the exposure taken in the worst case situation is longer than 1000 seconds. The maximum heat produced by any component in the light path is less than 42 °C. Temperatures less than 42 °C are considered harmless by the European Medical Device Directive 93/42/EEC. The da Vinci® Fluorescence Imaging Vision System was built to avoid extraneous light release and thus prevent accidental exposure to light.

19.4.2 Manufacture

The da Vinci® Fluorescence Imaging Vision System is manufactured by Intuitive Surgical, Inc. It includes the following components:

- An illuminator capable of providing visible / NIR fluorescence illumination / excitation via a flexible light guide cable, and the image processing required to generate simultaneous, real-time HD video and fluorescence images
- One flexible light guide cable
- A stereoscopic camera for visible / NIR fluorescence imaging.
- A surgical laparoscope optimized for visible / NIR fluorescence illumination and imaging

The da Vinci® Fluorescence Imaging Vision System is a component of the da Vinci® Surgical System. As such, the System includes:

- Medical grade System Vision Cart which includes a Medical Grade HD Video Monitor
- Medical grade Surgeon Console for teleoperation of the Surgical System
- Medical grade Patient Side Cart, whose arms hold both the teleoperated laparoscopic instruments as well as the camera / laparoscope assembly.

The da Vinci® Fluorescence Imaging Vision System is CE marked and has received numerous FDA 510K clearances for use in minimally invasive surgery (K124031, K101077, K141077)

19.4.3 Module Testing

Modules of the da Vinci® Fluorescence Imaging Vision System system are tested before final system assembly. The individual components are tested as per the manufacturing process. These tests include functional and performance tests as well as any proscribed electro-medical safety tests. All components bear the CE mark. The 3rd party medical grade accessories such as drapes are stand-alone certified medical device components and are not further tested.

19.4.4 System Testing

The da Vinci® Fluorescence Imaging Vision System is tested in a system configuration during manufacturing. Additional system level testing is carried out during standard compliance testing in product development.

19.4.5 Packaging and Unpacking

Systems components are packaged separately and are combined when ordered. Upon delivery to the participating medical center, the designated representative will sign for the equipment and arrange for its secure storage.

Intuitive Surgical certifies that:

(a) The mutual compatibility of the components and accessories that comprise the da Vinci® Fluorescence Imaging Vision System has been verified;

(b) The information for users supplied with the da Vinci® Fluorescence Imaging Vision System incorporates relevant instructions from the manufacturer; and

(c) The appropriate methods of internal control and inspection have been applied.

19.4.6 Installation

Upon delivery to the participating medical center, the da Vinci® Surgical System will be assembled and tested as per operating instructions. The unit will be assembled, tested for operation, and kept as a complete unit.

19.4.7 Operation

The da Vinci® Fluorescence Imaging Vision System is used to image OTL38 as follows:

OTL38 is administered 2 to 3 hours prior to scheduled surgery. The agent is rapidly distributed after IV administration and also rapidly eliminated from the circulation. Tissues containing high concentrations of folate receptors sequester the agent.

When illuminated by the NIR excitation light produced by the da Vinci® Fluorescence Imaging Vision System, the tissues containing OTL38 continue to fluoresce while the surrounding tissues become dark. The agent was shown to preferentially label folate-receptor-positive tumors in animal models. Tumors that contain folate receptors will bind OTL38, and the da Vinci® Fluorescence Imaging Vision System will cause the tumors to appear green in the augmented image displayed in the Surgeon Console and on the video monitor. In animal models, tumor-specific fluorescence was shown to last at least 8 hours after administration allowing for post-surgical tumor imaging. Although normal kidney proximal tubule cells contain folate receptors

and uptake of OTL38 in the kidneys is possible, the fluorescence signal in the kidneys is expected to be significantly lower than the tumor tissues (Ross 1994).

Investigators will employ the device in their procedures as outlined. Once the patient has undergone surgical preparation for a partial nephrectomy and the laparoscope has been inserted into the abdomen, images of the surgical area will be captured using both a fluorescence and white light examination prior to tumor excision. The images will be evaluated to determine the extent of tumor involvement and confirm these assessments with normal visual examinations. Surgery will be performed as per usual protocol/procedure, after which a follow-up image of the surgical field will be captured using fluorescence imaging. The remaining fluorescent tissues will be evaluated for possible tumor involvement and the surgical procedure will be finished as indicated.

19.4.8 Device Agreement

The da Vinci® Fluorescence Imaging Vision System is supplied for the purposes of a clinical investigation of OTL38 for intraoperative of renal cancer. While it is an FDA-approved device when used in the indications for which is it approved, when used with OTL38 it should be considered an investigational device not intended for the diagnosis or treatment of human disease. This device is only intended for use in conjunction with OTL38 in the context of the clinical trial. By my signature below, I certify that I understand this agreement and will comply with these stipulations.

Signature of Principal Investigator _____

Name of Principal Investigator _____

Investigative Site _____

19.4.9 Certification of Signed Agreement

The Sponsor hereby certifies that the da Vinci® Fluorescence Imaging Vision System will only be provided to qualified investigators in conjunction with the clinical protocol entitled, “Intraoperative folate targeted fluorescence in renal cell carcinoma.”

19.4.10 Institutional Review Boards

This instrument will be used as part of a controlled clinical trial. The IRB will be identified on Form FDA 1572 for each investigator engaged.

19.4.11 Institutions

Each institution to be used in the clinical trial will be identified on Form FDA 1572.

19.4.12 Sale of Equipment

The system will not be sold to centers but will be provided as part of the clinical trial.

19.4.13 Environmental Assessment

The Sponsor applies for categorical exemption from an Environmental Assessment based on 21 CFR 25.31.

19.4.14 Labeling for the Device

The da Vinci® Fluorescence Imaging Vision System contains labels as described in the Operator's Manual. The Sponsor will attach a label on each extended system cart identifying the system as:

da Vinci® Fluorescence Imaging Vision System

FOR INVESTIGATIONAL USE ONLY

This device is only to be used by qualified investigators in conjunction with the clinical protocol entitled, "Intraoperative folate targeted fluorescence in renal cell carcinoma"

Sponsor: IU

19.4.15 Operator's Manual

The instructions for use of the da Vinci® Fluorescence Imaging Vision System are contained in the Site Manual, Instructions for Use document. The Operator's Manual includes the following chapters and appendices:

- Health and Safety Compliance
- Unpacking and Set-up
- Controls and Indicators
- Operation
- Cleaning and Sterilizing
- Troubleshooting

19.4.16 Informed Consent Form

The informed consent form will be the same as that for entry into the clinical study.

19.4.17 Other Information for da Vinci® Fluorescence Imaging Vision System

19.4.17.1 Specifications

Table-1 VPI Specifications

Feature		Specification	
Light Sources	Spectrum	400 – 700 nm	Near infrared (NIR)
	Type	Light emitting diode array	NIR laser diode
Inputs/Outputs	Video output signals	HD-SDI, DVI, S-Video	
	HD format	1080i	
	Picture elements	1920 x 1080	
	Service port I/O	Ethernet (IEEE 802.3)	
Operator Controls	Power on/off	Front panel switch	
	Standby	Front panel button	
	White balance	Camera Head	
	White light/fluorescence mode	Surgeon Console / Vision Cart Button	
	Video display option	Vision Cart Button	
Operating Environment	Operating temperature	+10° to +30°C	
	Relative humidity	10 to 85% non-condensing	
Storage and Transport Environment	Humidity range (storage)	10 to 85%	
	Temperature range (storage)	-10° to +55°C	
	Humidity range (transport)	5 to 95%	
Physical	Dimensions	W 394 mm × H 140 mm × D 432 mm	
	Weight	13 kg	
Electrical Power	Voltage	100/120/230 VAC	
	Power frequency	50/60 Hz	
	Power consumption	400 VA	

Table-2 Camera Head Specifications

Feature		Specification
Optical	Image sensors	CCD HD sensor assembly
	HD format	1080i
	Aspect ratio	16:9
Physical	Dimensions	79 x 90 x 194 / 46 x 130 x 116 (mm)
	Weight	600 g
	Cable length	5.75 m
Environment	Operating temperature	+10° to 30°C
	Relative humidity	10 to 85% non-condensing
	Storage temperature	-10° to +55°C
	Sterilization	Sterile Drape

Table-3 12 mm Laparoscope Specifications

Feature		Specification
Optical	Viewing angle	0° and 30°
	Field of view	60°
	Resolution	HD compatible
	Transmission spectrum	Visible + NIR
Physical	Outer diameter	12 mm
	Working length	387 mm
Environment	Operating temperature	+10° to 30°C
	Storage temperature	-10° to +55°C
	Sterilization	Sterrad

Table-4 Light Guide Cable Specifications

Feature		Specification
Optical	Transmission spectrum	Visible Light and Near-Infrared
Physical	Fiber diameter	~ 5mm
	Length	~ 5.75m
Environment	Operating temperature	+10° to 30°C
	Storage temperature	-10° to +55°C

Table-5 Equipment Classification

Feature	Specification	
Type of protection against electric shock	Class I	as per IEC 60601-1
Degree of protection against electric shocks	CF-type	
Degree of protection against moisture	Ordinary	
Laser class	Class 3R	as per IEC 60601-1
Radio frequency emissions	Group 1, Class A	as per CISPR 11
Harmonic emissions	Class A	as per IEC 61000-3-2

Table-6 NIR Radiation and Source Characteristics

Feature		Specification
Apertures for NIR radiation emission		Laparoscope tip or light *guide (if endoscope* is detached)
Accessible NIR radiation (at the tip of the laparoscope)	Wavelength	808 ± 4 nm
Embedded laser source	Classification	Class 3R, invisible

19.4.17.2 References

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19.5 Appendix E

SPY Elite Technical Information

SPY Elite

Appendix A
Technical Information

Table 1: SPY Elite Device Laser Characteristics

Feature	Specification		
Medical device classification	Class II	as per US FDA classification	
Type of protection against electric shock	Class I	as per IEC/EN 60601-1	
	The system is isolated from the supply mains by an isolation transformer, such that the leakage current is less than the requirements of UL/IEC/EN 60601-1.		
Laser class	Class 3R	as per IEC/EN 60825-1	
	Wavelength	**Maximum Output**	**Divergence**
	805 nm	119 mW	40°
	650 nm	5 mW	<0.2°
	Complies with 21CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.		
Radio Frequency emissions	Group 1, Class A	as per CISPR 11 / EN55011	
Electromagnetic immunity	Class A	as per IEC/EN 60601-1-2	
Harmonic emissions	Class A	as per IEC/EN 61000-3-2	
Voltage fluctuations & flicker	Complies	as per IEC/EN 61000-3-3	

Table 2: SPY Elite Device Embedded Laser Source

Parameter	Specification
Wavelength	805 nm
Maximum Power Output	15000 mW
Beam Divergence	25°
Laser Class of Embedded Source	4

NOVADAQ

Technical Specifications

- Video Output:
  - Signal: Digital video
  - Standard: DVI-D (Single-link)
  - Mode: 1680 x 1050 @ 60 Hz non-interlaced
  - Connector: DVI-D (digital only)
- External data interface:
  - Standard: USB
  - Interfacing devices: Data storage devices
- Optical drive:
  - Mode: Read/Write
  - Media: DVD-R
- External power supply:
  - Voltgage: 120 V~
  - Frequency: 60 Hz
  - Power: 1000 VA
- Weight: 165 kg

Operating Conditions

- Temperature +15 °C to +30 °C
- Humidity 10 % to 85 % relative humidity, non-condensing
- Atmospheric Pressure: 94 kPa to 102 kPa (-100 ft to +2000 ft)

Storage Conditions

- Temperature +10 °C to +30 °C
- Humidity 10 % to 90 % relative humidity, non-condensing
- Atmospheric Pressure: 94 kPa to 103 kPa (-500 ft to +2000 ft)

Shipping Conditions

- Temperature -20 °C to +40 °C
- Humidity 10 % to 100 % relative humidity, condensing
- Atmospheric Pressure: 70 kPa to 103 kPa (-500 ft to +10000 ft)
- Allow time after transport for temperature and humidity to equalize before operating the device.

19.6 Appendix F

NCI Common Toxicity Criteria (Version 4.0)

Due to the size of the latest version of the Common Toxicity Criteria, copies of this appendix are not included with this protocol document.

An electronic copy is available on the CTEP web site, http://ctep.cancer.gov/reporting/ctc.html

19.7 Appendix G

Performance Status Scales/Scores

	ECOG or Zubrod		**Karnofsky**		**Lansky**
Score	Activity	Score	Activity	Score	Activity
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
		90	Able to carry on normal activity; minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly.
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.

2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.	60 50	Requires occasional assistance, but is able to care for most of his/her needs. Requires considerable assistance and frequent medical care.	60 50	Up and around, but minimal active play; keeps busy with quieter activities. Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	40 30	Disabled, requires special care and assistance. Severely disabled, hospitalization indicated. Death not imminent.	40 30	Mostly in bed; participates in quiet activities. In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.	20 10	Very sick, hospitalization indicated. Death not imminent. Moribund, fatal processes progressing rapidly.	20 10	Often sleeping; play entirely limited to very passive activities. No play; does not get out of bed.

19.8 Appendix H

DSMC Meeting Minutes Template

Meeting Minutes Form for DSMC

send to dsmc@iupui.edu or file in binder

Meeting Date:			
Team/Program: (include meeting sign in sheet)			
Protocol & Status (open/closed to accrual) (one protocol per sheet)			
PI:			
	Y	N	
Weekly and Monthly meetings should include discussion on data, dose levels, accrual numbers, deviation summaries and SAE reports (per IUSCC DSMP).			
Has accrual been reviewed and entered into Oncore?			
Have all SAE's been entered into Oncore?			
Is there documentation for study discontinuation?			
Have all deviations been entered into OnCore?			
Have study deviation summaries been reviewed by the team *(CTO continue to keep deviation logs signed by PI) ?*			
Record any dose limiting toxicities (DLT's) on this form for any phase I investigator initiated trial, HOG or on a multi-site trial in which IUSCC is the lead site.			
If any of your answers are "NO" please explain in the space below.			
*Notes			

This form is to be used for Investigator Initiated and HOG trials (High risk weekly, Moderate risk Monthly, Low risk quarterly)

19.9 Appendix I

OTL38 Pharmacy Dose Preparation Manual

Please reference the Pharmacy Dose Preparation Manual for this study which can be made available upon request.