Functional Microscale Organotypic Assays to Predict Patient Response to Anti-Angiogenesis Therapies

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Funding Sponsor: NIH

Protocol Revision History

Version Date	Summary of Revisions Made
20 Sep 2017	Original Version
25 Oct 2017	UWCCC PRMC requested modifications
03 May 2018	Blood samples will be collected in addition to the tissue samples by UWCCC TSB
22 April 2019	Added background information for additional PET/CT imaging for immune-based systemic therapies and included additional FDG PET/CT imaging time-points for immune-based systemic therapies
13 Oct 2020	Remote consent procedures; clarification of inclusion criteria

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LIST OF ABBREVIATIONS

AE	Adverse event
CAEPR	Comprehensive Adverse Events and Potential Risks
CDx	Companion diagnostic
CSRAs	Chemotherapy Sensitivity and Resistance Assays
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB/DSMC	Data Safety Monitoring Board/Committee
FDA	Food and Drug Administration
GE	General Electric
GFR	Glomerular filtration rate
HIPAA	Health Insurance Portability and Accountability Act
IEC	International Electrotechnical Commission
IRB	Institutional Review Board
IV	Intravenous
IND	Investigational New Drug
kg	kilogram
MBq	megabecquerel
mCi	millicurie
mGy	milligray
MicroC^3	microfluidic-cis-co-culture
mL	milliliter
mmol	millimole
mRCC	Metastatic Renal Cell Carcinoma
PACS	Picture Archiving and Communication System
PET	Positron Emission Tomography
PI	Principal Investigator
PSMA	Prostate-specific membrane antigen
RCC	Renal Cell Carcinoma
REM	Roentgen Equivalent Mammal
RF	Radio frequency
RPF	Radiopharmaceutical Production Facility
SAE	Serious Adverse Event
UP	Unanticipated Problem
UW	University of Wisconsin
UWHC	University of Wisconsin Hospital and Clinics

STATEMENT OF COMPLIANCE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

I agree to ensure that all staff members involved in this study are informed about their obligations in meeting the above commitments.

Principal Investigator: Steve Y. Cho, MD

Signed: _____ Date: _____

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PROTOCOL SUMMARY

Title	Functional Microscale Organotypic Assays to Predict Patient Response to Anti-Angiogenesis Therapies.
IND Sponsor-Investigator	Steve Y. Cho, MD
Co-Investigators	David Beebe, PhD E. Jason Abel, MD Christos Kyriakopoulos, MD KyungMann Kim, PhD Wei Huang, MD
Participating Site	University of Wisconsin-Madison
Patient Population	Patients diagnosed with metastatic clear cell RCC that are surgical candidates
Accrual Objective	This research will enroll 15 subjects
Study Design	Phase 2, single-center, open-label study
Study Duration	The estimated accrual period is over 3 years
Primary Objective	The primary objective of this research is to evaluate response to systemic therapy, including anti-angiogenesis and/or immune-based therapies, via 18F-DCFPyL PSMA-based PET/CT in patients with metastatic renal cell carcinoma and to compare qualitatively with conventional imaging response criteria (RECIST 1.1) and histopathological endpoints including isolation, enumeration and staining of Circulating Tumor Cells (CTC).
Secondary Objectives	1. Measure therapy response of patient specific uVESSEL model 2. Evaluate the predictive power and validate the uVESSEL model
Inclusion Criteria	• Patients diagnosed with locally advanced (≥cT3) or metastatic clear cell RCC, as proven by biopsy. • Adults, 18 years of age or older. • Surgical candidates who have clinical indication for nephrectomy and standard of care biopsy of metastatic disease followed by possible standard of care systemic anti-angiogenesis based treatment regimen • Have consented to participate in the UWCCC Biobank.
Exclusion Criteria	• Patients who have received prior RCC systemic therapies • Prior history of prostate cancer • Prior history of any other malignancy within the last 2 years, other than skin basal cell or cutaneous superficial squamous cell carcinoma that has not metastasized and superficial bladder cancer • Unable to lie flat during or tolerate PET/CT • Serum creatinine > 2 time the upper limit of normal
Treatment Description	^{18}F-DCFPyL whole body PET/CT scan administered at the following timepoints: 1) Prior to scheduled nephrectomy 2) After completion of systemic antiangiogenesis and/or immune-based therapy 3) When patient is refractory to treatment
Study Procedures	• ^{18}F-DCFPyL whole body PET/CT scan • Review of relevant imaging and medical record information
Statistical Considerations	A total of 15 patients will be recruited into the clinical study. This is based on the feasibility and availability of suitable patients over the funding period with

	approximately five patients per year. With 15 patients, the mean of quantitative endpoints such as tumor size and SUV parameters will have standard error that is 0.258 times the sample standard deviation, and the width of a 95% confidence interval for the probability of binary qualitative endpoints such as objective response will be less than 0.253.

1.0 INTRODUCTION

Renal cell carcinoma (RCC) is the third most common genitourinary malignancy in the United States with ~64,000 new cases and ~14,000 deaths per year (Siegel 2017). Despite the increase in the number of treatment options, median overall survival for patients with metastatic RCC (mRCC) remains at less than 2 years (Heng 2013).

The purpose of this research is to build on previous success with using patient's own cells to predict their response to first line therapies for the treatment of multiple myeloma (MM). This research represents a transdisciplinary approach that leverages the recent development of a practical, functional, and adaptable *in vitro/ex vivo* model of RCC vascularization, coupled with novel prostate-specific-membrane antigen (PSMA)-based PET imaging of tumor neovasculature, to evaluate the model's ability to predict an individual patient's response to anti-angiogenic therapeutics.

2.0 RATIONALE

Recently, David Beebe's Laboratory has shown in a retrospective study that harnessing the micro scale to retain critical multi-cellular signaling, e.g. stromal-cancer, allows the use of a patient's own cells to predict that patient's response to first line therapy, i.e. bortezomib, in multiple myeloma (MM) (Pak 2015). There are multiple cell types in the bone marrow microenvironment, including bone marrow stromal cells (BMSCs), macrophages, osteoclasts, and other immune cells, which secrete factors, e.g. cytokines, known to regulate signaling pathways that may contribute to chemotherapy resistance in MM (Asimakopoulos 2013, Hideshima 2007, Hope 2014, Kim 2012, Markovina 2010). Therefore, in the Pak (2015) study, Beebe's lab used a simple, yet novel, micro scale co-culture system to create a companion diagnostic (CDx) to measure the drug response of cancer cells taken from the patient (Fig. 1, top). We will work together with David Beebe and his lab to adapt and apply an analogous approach (Fig. 1, bottom) using a micro scale organotypic co-culture model (subsequently referred to as uVESSEL) to predict response to anti-angiogenic therapies in RCC.

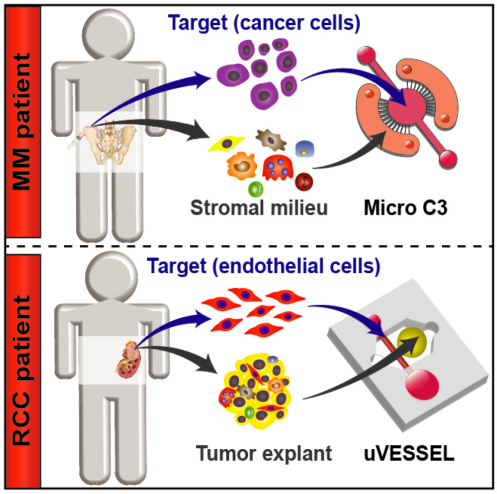


Fig. 1 - Top - Patient specific co-culture system used to predict response to first-line therapy in MM (Pak 2015). Bottom - Analogous approach for RCC with endothelial cells as target (and readout) for response to anti-angiogenic drugs.

RCC is the third most common genitourinary malignancy in the United States with ~64,000 new cases and ~14,000 deaths per year (Siegel 2017). Clear cell RCC accounts for up to 85% of all RCCs and is different from MM in important ways. Clear cell RCC tumors are highly vascularized with blood vessels comprising approximately 25% of the tumor volume, increasing the supply of nutrients and driving tumor growth and metastasis. These tumor-associated blood vessels are enlarged, lack hierarchy, have a leaky endothelial layer, have abnormal disconnected sprouts, and have up-regulated soluble factor secretion when compared to their normal counterparts (Dudley 2012). Thus, endothelial cells and neovascularity are key components of the RCC tumor biology and, therefore, anti-angiogenic drugs have been the

preferred approach to treat RCC. Anti-angiogenic drugs are designed to target existing blood vessels and prevent the generation of new blood vessels in addition to normalizing the vessels within the tumor to improve the delivery of chemotherapeutics in a combination therapy (Hida 2016). Thus, while cancer cells were the therapeutic target in MM, endothelial cells are the therapeutic target in RCC (Fig. 1). Yet as decades of testing with Chemotherapy Sensitivity and Resistance Assays (CSRAs) attest (Editorial 2013) and recent American Society of Clinical Oncology (ASCO) guidelines confirm (Burstein 2011), examining the target cell in isolation is insufficient to predict patient response reliably. However, David Beebe's recent MM work (Pak 2015), as well as work by Ramamoorthy (2015), has shown that inclusion of patient specific cancer-stroma interactions can enable accurate prediction of drug response. In this study, we will include the tumor milieu via incorporation of tissue explants in our micro scale RCC CDx (Fig. 1, bottom).

Despite increased clinical options, overall outcomes remain poor in RCC highlighting the need for predictive assays - Over the last decade, treatment options for mRCC have increased with the development of targeted agents that inhibit tumor angiogenesis. Unfortunately, median overall survival for mRCC patients remains less than 2 years (Heng 2013) and there remains an urgent need to improve treatment of mRCC patients. The first line agents pazopanib and sunitinib are multi-targeted small molecule, tyrosine kinase inhibitors (TKI) that inhibit a broad range of receptors for pro-angiogenic factors including VEFGR, PDGFR, and c-kit among others. These therapies are thought to act primarily or exclusively on tumor endothelial cells, decreasing tumor blood supply (Huang 2010). Axitinib and cabozantinib have similar (and also distinct) mechanisms of action, but clinically these agents are used as a second-line therapy only when primary agents fail. Importantly, wide variation in treatment responses to different agents has been demonstrated in clinical trials with anti-angiogenic therapies (Heng 2013). Currently, no biomarkers are available to predict which therapy will provide the best response to individual tumors and a "trial and error" approach is used to select initial treatments for mRCC patients. This standard paradigm is based on randomized clinical trial data demonstrating better clinical outcomes. The disadvantages of this empiric (and population-based) approach to selecting initial treatments in mRCC is also magnified by the rapid development of ten FDA approved therapies in ten years. This rapid expansion of new agents quickly outpaced the ability to evaluate these treatments in clinical trials. In 2017, clinicians and patients now have many choices for systemic treatment of mRCC, but desperately need tools for selecting the best treatments for individual patients. A better approach would be to test each patient with a CDx to determine which of the many available drugs is most appropriate for that patient, resulting in precision medicine for treatment of patients with mRCC.

As discussed above, the molecular pathogenesis of RCC is intrinsically linked to angiogenesis and the use of anti-angiogenic drugs. Our micro scale model will recapitulate patient vasculature and we need a method to measure vascularization in vivo in patients in order to evaluate the predictive value of our model. Current conventional imaging response criteria originally developed to assess response to chemotherapy using computed tomography (CT), such as RECIST1.1, are limited for determining treatment response to anti-angiogenesis agents (Ammari 2014). In order to monitor response in patients we can exploit the highly vascularized tumors in RCC, using a positron emission tomography (PET) radiotracer targeting the tumor neovasculature to reliably image RCC metastatic lesions. A new PET radiotracer for imaging angiogenesis targets the cell surface protein, prostate-specific membrane antigen (PSMA), which, despite the specificity implied by its name, is highly expressed on the tumor neovasculature of a number of solid tumors including a variety of RCC subtypes but most pronounced in clear cell RCC (Baccala 2007, Al-Ahmadie 2008). Recent studies by ourselves and others have reported

PSMA-based PET detection of metastatic RCC (Siva 2017, Gorin 2017, Rhee 2016, Rowe 2015 & 2016). The development of PET imaging compounds targeted to the vascular endothelium allows direct imaging of tumor neovasculature, avoiding the limitations of indirect methods such as traditional contrast enhanced imaging. Thus, PSMA PET imaging addresses a major unmet clinical need in metastatic RCC for a reliable non-invasive functional imaging method for metastatic tumor detection and response to therapy, especially for anti-angiogenesis treatments.

In addition to anti-angiogenesis agents, immunotherapy has also been shown to play an important role in the treatment of patients with metastatic RCC. Nivolumab is an anti-programmed cell death 1 (PD-1) monoclonal antibody that was approved by FDA in 2015 based on a phase III clinical trial that showed improvement in survival compared to the mTOR inhibitor everolimus in patients whose disease had developed resistance to anti-angiogenesis therapies (Motzer 2015). More recently, combination immunotherapy with nivolumab and ipilimumab, which is an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) monoclonal antibody, demonstrated improvement in survival compared to sunitinib (Motzer 2018) and eventually became the preferred standard-of-care first-line treatment for patients with metastatic RCC. Finally, two additional trials that were published in March of 2019 showed that the combination of axitinib with anti-PD-1 immunotherapy (pembrolizumab and avelumab respectively) [Rini 2019; Motzer, 2019] showed superiority compared to sunitinib and are currently pending approval by FDA as first-line therapies for metastatic RCC. In this rapidly evolving field, it is unclear how the most recent studies will impact the care of these patients, however systemic therapy with anti-angiogenesis agents will continue to play an important role.

3.0 OBJECTIVES

3.1 Primary Objective: To evaluate response to systemic therapy, including anti-angiogenesis and/or immune-based therapies, via 18F-DCFPyL PSMA-based PET/CT in patients with metastatic renal cell carcinoma and to compare qualitatively with conventional imaging response criteria (RECIST 1.1) and other histopathological endpoints including isolation, enumeration and staining of Circulating Tumor Cells (CTC).

Response of systemic immune-based and/or anti-angiogenesis therapies will be quantified using PSMA-based PET imaging using a novel agent,^{18}F-DCFPyL, as a non-invasive imaging biomarker of tumor neovasculature to functionally monitor renal cell cancer neovasculature in patients undergoing systemic anti-angiogensis therapy. PSMA PET will be compared with response to anti-angiogenesis therapy using conventional imaging computed tomography(CT)-based RECIST1.1 criteria (Eisenhauer 2009) as well as histopathological endpoints (tumor vascular density, immunohistochemical staining for PSMA and neovascularization (CD105, CD31). Whole body PSMA PET/CT scans will be obtained at baseline, following adjuvant anti- angiogenic therapy and when the patient becomes refractory to treatment.

The rationale and time points for obtaining PET scans is planned with respect to the typical natural history of metastatic RCC. This project will obtain information from tumors that are responding to anti-angiogenesis therapy and those resistant to treatment.

PET1 is obtained pre-surgically to establish the baseline tumor PET and CT characteristics prior to

surgical removal of the primary renal tumor and metastatic sites (e.g. LNs). Surgery technique is not altered for this study.

PET2A is obtained post-surgery and prior to start of first line standard-of-care (SOC) systemic therapy, either immune-based and/or anti-angiogenesis therapy. If the first line SOC systemic therapy is an immune-based therapy, a PSMA-based ^{18}F-DCFPyL PET/CT will be obtained as PET2A and PET2B imaging time points, with potential PET3A and PET3B imaging as possible intended anti-angiogenesis therapy. The purpose of PET2 imaging is to establish a new baseline PET before first line SOC systemic therapy commences, given that post-surgical healing is a complex process that also includes angiogenesis and post-surgical inflammatory changes with potential changes in PET imaging. PET2 is necessary to characterize the existing metastatic tumors and any metastatic tumors which develop between PET1 and beginning systemic therapy. Clinical imaging with standard imaging is also obtained at this time as per standard care.

PET2B is obtained at 12-16 weeks from start of first-line SOC systemic therapy, either immune-based and/or anti-angiogenesis therapy (with associated SOC conventional diagnostic chest/abdomen/pelvis CT) to evaluate mRCC tumors response to treatment. This time point is chosen given that less than 10% of patients will have progressive disease on SOC conventional imaging at their initial evaluation.

PET3A and P**ET3B** are obtained as follows, if the first line SOC systemic therapy did not include an anti-angiogenesis therapy and if this new systemic therapy includes an anti-angiogenesis therapy:

> **PET3A** is obtained prior to start of additional systemic therapy including anti-angiogenesis therapy. The purpose of PET3A imaging is to establish a new baseline PET before this systemic therapy commences. PET3A is necessary to characterize the existing metastatic tumors and any metastatic tumors which develop between PET2B and start of this systemic therapy. Clinical imaging with standard imaging is also obtained at this time as per standard care.
>
> **PET3B** is obtained at 12-16 weeks from start of this new systemic therapy including anti-angiogenesis therapy (with associated SOC conventional diagnostic chest/abdomen/pelvis CT) to evaluate mRCC tumors response to treatment.

PET4 is obtained at clinical progression per (RECIST 1.1) or 2 years following initial systemic therapy, whichever occurs first. In patients with progressive disease, PET imaging will be used to evaluate changes in index lesions. In recent review (NEJM 2017) of clinical trials for first line mRCC therapies, the median time to progression on therapy is less than one year (range 3.1-11 months), with median OS about 2 years (range 7.3- 29 months). (Krajewski 2014 and Choueiri 2017).

3.2 Secondary Objectives:

3.2.1 Measure therapy response of patient specific uVESSEL model

Patient-derived uVESSEL models (from primary & metastatic sites) and incorporating patient-derived immune cells will be tested for response to anti-angiogenic therapies including pazopanib, sunitinib, cabozantinib, and axitinib. Response will be quantified via multiple endpoints, e.g. vessel sprouting, PSMA expression, cell death.

3.2.2 Evaluate the predictive power and validate the uVESSEL model

While a number of novel biomimetic approaches show promise in their ability to recapitulate physiologically relevant behavior, clinical validation is needed to determine if such approaches are predictive. Using the clinical, pathological and imaging endpoints under 3.1, we will develop predictive models using responses to uVESSEL model above. More specifically we will use the objective response (dichotomous endpoint of yes or no) measured using PSMA-based PET/CT as a dependent variable in a logistic regression model with response to uVESSEL model from above as an independent predictor to segregate uVESSEL responses into two groups corresponding to responders and non-responders to anti-angiogenic therapies.

3.2.3 Evaluate the predicative power and validate in-vitro immune therapy models

At the moment we are expanding our uVESSEL patient-specific organotypic models (Fig. 2) to contain more cell types from the tumor microenvironment (cancer epithelial, endothelial, immune, stromal) in similar proportions as found *in vivo*.

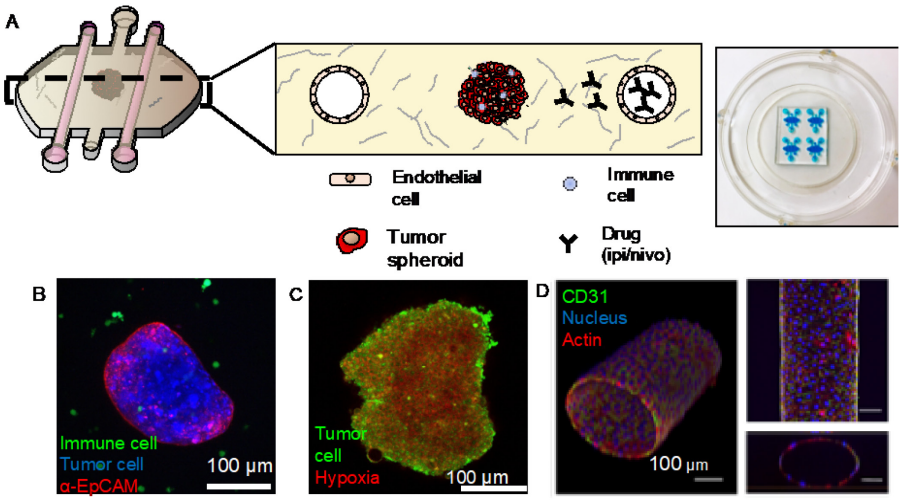


Fig. 2. A) Left: model scheme showing a tumor spheroid flanked by 2 endothelial lumens. Right: a 4-microdevice array. Confocal images of B) a tumor spheroid (blue) and immune cells (green). C) tumor organoid (green) with hypoxic core (red). D) lateral endothelial lumen.

Patient tissue can be disaggregated, specific cell types isolated and placed in the uVESSEL model (Bischel 2014) (Jimenez-Torres et al 2019). Tumor spheroids can be created from a mixture of tumor epithelial cells and immune cells, to mimic tumor-infiltrating leukocytes, embedded in a hydrogel matrix (Sung 2014). Tumor spheroids model solid tumors and replicate the hypoxia and nutrient starvation observed *in vivo* in RCC (Fig. 2). Hypoxia influences the microenvironment by significantly suppressing the anti-tumor response by increasing regulatory and immunosuppressive immune cell phenotypes and reducing cytotoxic lymphocyte functions (Noman 2015). Likewise, the uVESSEL model allows for the creation of lumen structures within a hydrogel matrix that can be lined with endothelial cells to form microvessel structures, mimicking the highly vascularized nature of RCC (Jimenez-Torres 2019). Thus, we can create a carefully controlled model tumor microenvironment made entirely of primary cells all from the same patient (Fig. 2) that can be treated with various immune checkpoint inhibitors, TKI's or combinations thereof with readouts examining tumor growth, immune cell phenotype and function and angiogenesis. Our functional endpoints include spheroid size variation/viability, as well as the angiogenic sprouting endpoint already developed for TKI predictive value. Both outputs can be expressed as a dichotomous endpoint of responders/non-responders for the validation of uVESSEL performance with patient response as observed in PET/CT.

4.0 RESEARCH DESIGN AND METHODS:

Study Design: This study is designed as a single cohort study without randomization or stratification.

Study population: A total of 15 patients diagnosed with clear cell RCC will be enrolled in this study. Achieving the objectives outlined above will require the collection of tissue from the UWCCC Biobank, the review of subject medical records, and subject participation in up to four research visits lasting approximately 2 – 3 hours.

Inclusion Criteria:

- Patients diagnosed with locally advanced (≥cT3) or metastatic clear cell RCC, as proven by biopsy.
- Adults, 18 years of age or older.
- Surgical candidates who have clinical indication for nephrectomy and standard-of-care biopsy of metastatic disease followed by possible standard of care systemic anti-angiogenesis based treatment regimen
- Have consented to participate in the UWCCC Biobank.

Exclusion Criteria

- Patients who have received prior RCC systemic therapies
- Prior history of prostate cancer
- Prior history of any other malignancy within the last 2 years, other than skin basal cell or cutaneous superficial squamous cell carcinoma that has not metastasized and superficial bladder cancer
- Unable to lie flat during or tolerate PET/CT
- Serum creatinine > 2 times the upper limit of normal

Subject identification and recruitment

GU Oncology staff will identify patients who meet the eligibility criteria and will introduce them to this research opportunity. Those interested will be referred to a study team member who will review the purpose of the research as well as details about participation.

Informed Consent and Subject Information

The consent process will occur prior to the administration of research PET/CT procedures. Potential subjects will meet with a study team member to review the information in the consent form including study procedures, risks associated with participation, alternatives to participation and whom to contact for additional information. Any questions will be addressed prior to the start of any study procedures and all subjects will be reminded that participation is optional and they can change their mind at any time.

The consent process will occur prior to the administration of research procedures. During the COVID-19 pandemic, consent procedures will be conducted by phone, when possible, to minimize face-to-face contact subjects have with the research team. A copy of the consent form will be mailed or emailed to subjects prior to the scheduled consent phone call. If emailed, the consent form will be encrypted. A study team member will call potential subjects at the scheduled time to review the information in the consent form including study procedures, risks associated with participation, alternatives to participation and whom to contact for additional information. Any questions will be addressed during the course of the phone call and subjects will be encouraged to contact the study team with any questions or concerns

they might have at any time. Upon completion of the consent process, a copy of the signed consent form will be provided in one of the following ways depending on each subject preferences and capabilities:

- Electronic signature will be provided using DocuSign
- Subject will be asked to scan a copy of the entire consent document and email it back to the study team.
- Subject will be asked to take a photo of the signature page and email a copy to the study team.
- If subjects are unable to provide an electronic copy of the signed consent form, they will be asked to bring a copy of the form to the research visit.

5.0 STUDY PROCEDURES

This project was designed to seamlessly integrate with the current pathway and standard of care both surgically and with medical oncology treatments. The patient will be evaluated in Dr. Abel's clinic and discussed with Drs. Kyriakopoulos, Cho and Huang in the weekly multi-disciplinary conference from 12-1 pm every Thursday. Suitable patients will be invited to participate in this research by someone involved in their care. Those interested in participating will be referred to a member of Dr. Cho's research team. Patients enrollment in the UWCCC Biobank program will be conducted in accordance with the Biobank protocol.

Subjects will obtain standard of care (SOC) labs and pre-operative imaging as well as research PSMA-PET imaging prior to surgery. Surgery will proceed as per SOC and will not have extra procedures specific to this project. Patients will have time to recover following surgery and receive SOC imaging. Metastatic RCC patients will havea 2nd PSMA-PET (PET2A) greater than 4 weeks post-surgery and prior to initiation of systemic therapy. Patients with locally advanced disease will not have PET2A until metastatic RCC identified with SOC imaging. The rationale for the second PSMA PET prior to start of systemic therapy (PET2A) is to establish a new baseline PET. We want to establish any potential post-surgical PET findings from PET1 to PET2A (ex: post-surgical PET changes, change in PSMA PET uptake at sites of remaining measurable disease, new potential lesions). This will be followed by a third PSMA PET (PET2B) obtained at 12-16 weeks from start of first-line SOC systemic therapy, either immune-based and/or anti-angiogenesis therapy (with associated SOC conventional diagnostic chest/abdomen/pelvis CT) to evaluate mRCC tumors response to treatment. This time point is chosen given that less than 10% of patients will have progressive disease on SOC conventional imaging at their initial evaluation.

If the first line SOC systemic therapy did not include an anti-angiogenesis therapy and if this second systemic therapy includes anti-angiogenesis therapy, an additional 4th and 5th PET imaging will be obtained as PET3A and PET3B. PET3A is obtained prior to start of additional systemic therapy including anti-angiogenesis therapy, to establish a new baseline PET before this systemic therapy commences, to characterize the existing metastatic tumors and any metastatic tumors which develop between PET2B and start of this systemic therapy. PET3B is obtained at 12-16 weeks from start of this second systemic therapy including anti-angiogenesis therapy (with associated SOC conventional diagnostic chest/abdomen/pelvis CT) to evaluate mRCC tumors response to treatment.

Following recovery from surgery the patients will initiate systemic therapy as per SOC. The patients will receive systemic therapy with an antiangiogenic agent as per first-line or later SOC therapy. There are currently 4 oral antiangiogenic agents approved by FDA for the treatment of patients with metastatic RCC (pazopanib, sunitinib, cabozantinib, axitinib) and patients on this study will receive one of those 4 agents as per physician's preference.

Study procedures will include a tissue analyses as well as the use of PET/CT imaging to monitor subject's clinical response to treatment. For tissue, immune cell and CTC analyses, investigators will be working with the UWCCC Biobank staff to acquire blood samples and tissue samples donated from the kidney with primary tumor, lymph nodes, and any other metastatic site from which tissue was collected.

Subjects will be asked to complete four to six research visits that will include the administration of a PSMA PET/CT exam. There will be an initial baseline ^{18}F-DCFPyL PSMA PET/CT (PET1) prior to nephrectomy and SOC biopsy of metastatic sites as clinically indicated, followed by a second ^{18}F-DCFPyL PSMA PET/CT (PET2A) prior to start of systemic anti-angiogenesis and/or immune-based therapy. A third on-treatment ^{18}F-DCFPyL PSMA PET/CT (PET2B) will be performed at 12-16 weeks from start of systemic therapy (with associated SOC diagnostic chest/abdomen/pelvis CT). If system The fourth (or sixth if there is PET3A and PET3B) ^{18}F-DCFPyL PSMA PET/CT (PET4) will be performed at the time of disease progression as determined by conventional imaging such as CT or MRI; or two years after initiation of therapy (whichever one comes first).

Blood and Tissue Collection

We are collaborating with the UWCCC Translational Science BioCore (TSB) for sample collection as this research is consistent with their mission "to facilitate cancer-related basic science and translational research within the UWCCC through the integration of human biospecimen collection and storage with associated histology, molecular, morphometric and consultative services". The TSB team is uniquely equipped to appropriately identify and consent patients and to collect and properly handle/process the samples without disrupting the clinical workflow. As a result, this research will only target patients that have previously consented to allow additional and residual tissue samples to be included in the UWCCC Biobank. Biobank personnel will provide blood and the sample(s) collected from the kidney with primary tumor, lymph nodes, and any metastatic sites that are excised.

PET/CT Imaging

Each PET/CT research visit is estimated to take 2-3 hours to complete. Women of childbearing potential will be administered a pregnancy test prior to the start of imaging procedures. Subjects will be asked to fast for 4 to 6 hours and to stay well hydrated prior to each visit. Upon their arrival to the WIMR facilities, an IV will be placed to allow injection of the PET tracer. After the IV administration of less than 9 mCi of ^{18}F-DCFPyL radiotracer by slow IV push, subjects will be required to sit quietly for 1-hour to allow for the biodistribution of the tracer. After approximately 50 minutes, subjects will be given the option of using the restroom prior to being positioned in the PET/CT scanner. Once positioned, a whole body (defined in this protocol as vertex-of-skull to mid-thigh) PET scan will be obtained with corresponding low-dose non-IV contrast CT imaging with the PET/CT obtained for PET attenuation correct and anatomic co-registration.

A member of the study team will follow-up with all subjects approximately 1 to 3 days after their PET/CT exam by phone to inquire about delayed adverse events. If the subject is experiencing any late occurring adverse events, they will be asked to return to the research clinic or urology clinic for a follow-up exam.

Review of Medical Records

Medical records will be reviewed to obtain medical history including physical exams, medications (all concomitant medications before and after each of the PET/CT scans, treatment medications for RCC), laboratory tests (metabolic chemistry, hematology, pathology), imaging results (all available CT, MRI, ultrasound, PET/CT) and information related to the diagnosing, treatment, or outcomes of the study subject's diagnosis, treatment and clinical follow-up up to two years from the time of initiation of systemic anti-angiogenesis therapy.

Remuneration

Subjects will receive $50 per scan, for a total of up to $300, as compensation for their participation in this study.

Withdrawal/Termination from Study

Reasons for withdrawal or termination

Enrolled patients will be removed from the study in the following circumstances:

- The patient does not complete the imaging examinations per study protocol
- The subject withdraws consent
- Exclusion criteria are discovered after registration but prior to the research examinations
- The PI or co-investigators determine it is within the best interest of the subject to remove them from the research.

Study Procedures Table

Study Procedures	Screening[1]	Baseline Prior to surgery	VISIT 2a and 2b Prior to and during 1st systemic therapy	VISIT 3a and 3b Prior to and during 2nd systemic therapy	VISIT 4[2]
Informed Consent[5]	X				
Clinical History	X				
PET/CT Exam		X	X[4]	X[4]	X
Tissue Collection			X[3]		

1. Screening visit may be completed on the same day as the baseline visit
2. To be done at the time of disease progression or within 2 years, whichever occurs first.
3. Tissue collection will occur post SOC surgery and will be coordinated with the UWCCC TSB.
4. PET/CT scan obtained prior to systemic therapy (immune-based and/or anti-angiogenesis therapy) and at 12-16 weeks into therapy
5. During the COVID-19 pandemic, consent procedures will be conducted by phone, when possible, to minimize face-to-face contact subjects have with the research team. An encrypted copy of the consent form will be mailed or emailed to subjects prior to the scheduled consent phone call.

6.0 RISKS AND BENEFITS

PET:
Detailed organ dosimetry derived from four representative patients is included in the table below. Of note, the effective dose from ^{18}F-DCFPyL was 0.0165 mSv/MBq or 6.1 mGy (0.61 rem) for an injected dose of 370 MBq (10 mCi), or 5.49 mGy or 5.49 mSv (0.549 rem) for 333 MBq (9 mCi) which is the highest dose administered for each scan in this study.

Dosimetry:
Highest radiation dose was estimated for the kidneys (0.0945 mGy/MBq) followed by urinary bladder wall (0.0864 mGy/MBq), submandibular glands (0.0387 mGy/MBq) and liver (0.0380 mGy/MBq). The mean absorbed dose to the bone marrow was 0.01 mGy/MBq. In comparison to the published data on our first generation agent, ^{18}F-DCFBC, ^{18}F-DCFPyL shows significantly lower (under 50 %) doses in most radiosensitive organs such as the thymus, ovaries, red marrow and osteogenic cells. Less radiation dose was also measured in lower large intestinal wall, small intestine, stomach wall, lungs, muscle and skin. Radiation dose from the two radiotracers was similar (within +/-50 %) in the gallbladder wall, upper large intestinal wall, spleen, testicles, thyroid, and pancreas. Radiation dose from ^{18}F-DCFPyL was higher in the adrenals, kidneys, liver, pancreas, spleen, and bladder wall.

Organ	Absorbed Dose (mGy/MBq)
Adrenals	3.11E-02
Brain	2.19E-03
Breasts	4.57E-03
Gallbladder Wall	1.44E-02
Heart Wall	1.29E-02
Kidneys	9.45E-02
Lacrimal Glands	3.50E-02
Lens	1.25E-03
Liver	3.80E-02
LLI Wall	1.05E-02
Lungs	1.08E-02
Muscle	6.32E-03
Osteogenic Cells	9.58E-03
Urinary Bladder Wall	8.64E-02

Organ	Absorbed Dose (mGy/MBq)
Ovaries	8.89E-03
Pancreas	2.44E-02
Parotid Glands	2.68E-02
Red Marrow	1.04E-02
Skin	4.05E-03
Small Intestine	9.13E-03
Spleen	1.85E-02
Stomach Wall	1.16E-02
Submandibular Glands	3.87E-02
Testes	1.01E-02
Thymus	5.56E-03
Thyroid	8.56E-03
ULI Wall	1.67E-02
Uterus	1.15E-02

CT:
Risks associated with CT include exposure to 10.0 mSv of radiation from the low-dose scan required for attenuation correction calculations. Thus, the total whole body effective dose equivalent for a PSMA PET/CT is approximately 15.5 mSv (5.5 mSv from PSMA-PET plus 1.0 mSv from CT). This corresponds to approximately 4 years of natural background radiation. The total whole body effective dose equivalent for 2 and 3 PSMA PET/CT scans performed over a one year time period is 31.0 mSv and 46.5 mSv, respectively, and remain below the annual occupational dose limit for radiation workers (50 mSv) in a one year time period. If a patient receives a 4th or 5th PSMA PET/CT scan during a one year time period due to inclusion of PET3A and 3B timepoints for PET/CT imaging at time of 2nd systemic therapy, the

total whole body effective dose equivalent will be 62 mSv and 77.5 mSv, respectively. The PET4 timepoint PSMA PET/CT will be performed at the time of tumor recurrence or two years after initiation of therapy (whichever one comes first). (Median OS for metastatic RCC patients is approximately 24 months.)

7.0 ADVERSE EVENTS AND SAFETY ISSUES

Definition of adverse events and potential risks: The term "adverse event" is defined in the International Conference on Harmonization Guideline for Good Clinical Practice as follows: "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product".

Medical conditions of a subject that exist prior to receiving the investigational imaging tracer will be recorded as medical history. After administration of the investigational imaging tracer, all new medical conditions are considered adverse events. Adverse events occurring in this trial will be recorded in data collection forms, regardless of whether or not they are thought to be associated with the investigational imaging tracer throughout the study period. However, the Investigator also will assess the possible relationship between the adverse event and the investigational imaging tracer.

For the purposes of this protocol, an adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness, abnormal laboratory finding) that emerges or worsens relative to pre-imaging baseline. We anticipate minimal adverse effects or toxicity with the investigational imaging tracer ^{18}F-DCFPyL in this study.

Definition of Serious Adverse Events (SAE), SAE list: Any adverse drug experience, occurring at any dose that results in any of the following:

- Death
- Is life-threatening (an event in which the patient was at immediate risk of death; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires or prolongs inpatient hospitalization
- Results in a persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and/or may require intervention to prevent one of the outcomes listed.

All other AEs will be treated as "non-serious" and are described in the final study report. An SAE that is definitely, probably or possibly related to the investigational imaging tracer will be reported immediately (within 24 hours of its determination) by telephone or fax by the Principal Investigator.

AE Characteristics: Toxicity will be graded according to the NCI Common Toxicity Criteria (CTCAE), version 4.03 (Appendix A; which can also be accessed and downloaded via the website: http://ctep.cancer.gov/reporting/ctc.html. The Investigators will monitor the occurrence of adverse events during the course of each patient in the study. The Investigators need to assess whether there is a reasonable possibility that the study imaging agent caused or contributed to an AE using the following criteria as a guide:

- Definite: The adverse event is clearly related to the investigational agent.
- Probable: There is a clinically plausible time sequence between the onset of the AE and administration of imaging tracer. The AE is unlikely to be caused by the concurrent/underlying illness, other drugs, or procedures.
- Possible: There is a clinically plausible time sequence between the onset of the AE and administration of imaging tracer, but the AE could also be attributed to the concurrent/underlying disease, other drugs, or procedures. "Possibly" should be used when the administration of the study imaging tracer is one of several biologically plausible causes of the AE.
- Unlikely: The adverse event is doubtfully related to the investigational imaging tracer.
- Not Related: Another cause of the AE is the most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the AE and the investigational imaging tracer; and/or a causal relationship is considered biologically implausible.

If any adverse event is considered to be definitely, possibly or probably related to the investigational imaging tracer, that event will be followed until resolution or until it is deemed chronic or irreversible by the Investigator. If an event is unlikely or not related to the investigational imaging tracer, the event will not be reported.

All AE will be documented and recorded in the medical record and will include:

I. Specific reaction according to NCI Common Toxicity Criteria, version 4.0.
II. Duration of the reaction

III. Severity/grade according to NCI Common Toxicity Criteria, version 4.03. If the adverse event is not addressed by the Common Toxicity Criteria, the severity should be rated as mild (grade 1), moderate (grade 2), severe (grade 3), or life threatening (grade 4).
IV. Relation of reaction to study agent
V. Management of reaction, including any interruption or dose modification of the study drugs.

Event Severity: In addition to classifying an adverse event as serious or non-serious, the Investigator and/or designated personnel will grade the adverse event to describe The Maximum intensity of the event. Specific criteria for the intensity of adverse events are described in the Common Toxicity Criteria (CTC: National Cancer Institute). Investigators should follow these criteria for grading and reporting adverse events.

An event may be severe, but may not be serious (e.g., severe headache). The severity of an adverse event will be assessed by study personnel using their best clinical judgment and includes but is not limited to taking into account the patient's past history, the patient's chart, interviews with the patient and

caregivers, and direct observation. The Investigator will also be asked to assess the possible relationship between the adverse event and the administration of the investigational imaging tracer.

Adverse Event Reporting: Per 21CFR312.32(c), all adverse events deemed both serious and unexpected associated with the use of the drug must be reported to the FDA and to all participating investigators as soon as possible and in no event later than 15 days.

The FDA will be notified of any unexpected fatal or life threatening experience associated with the drug as soon as possible but in no event later than 7 calendar days. The UW HS-IRB will be notified in accordance with posted institutional policy.

Adverse Events: Serious and Non Serious Adverse events will be recorded, regardless of whether or not they are thought to be related to the investigational imaging tracer. While all adverse events will be tabulated and reported in the study final report, serious adverse events will be reported in the course of the trial. Adverse events that meet criteria of a serious adverse event listed above will also be recorded and reported.

The Investigator will inform the IRB of all adverse events attributed to the investigational imaging tracer in accordance with posted institutional policy.

8.0 PRIVACY AND CONFIDENTIALITY PROTECTIONS

Research procedures will be conducted in private areas and we are only collecting the minimum amount of information necessary to conduct the study. No sensitive data (HIV status, illicit drug use) will be collected. Identifiable records will only be available to study team members.

Data are not being shared outside of UW except through publications, in which case the data do not identify individual subjects. Study data will be managed by the PI and co-PIs. Data will not be placed on unencrypted laptop computers. Coded electronic data will be kept on the secure network drives. A separate file linking codes to subject identity will be kept in the same manner, in a folder separate from study data. At the end of 7 years study data will be de-identified by destroying the link to identifiable information. Until that time data will remain coded and the file linking codes to subject identity kept as well.

All samples and images will be given a unique study number that cannot be linked to the patients' medical records in any way. Imaging research images will be stored on PACS under this study number only. The investigators will have access to the patients' electronic medical records that are relevant to this study. Study records will be kept for seven years. Study data will be managed by the PI and co-PI.

Subject's identifiable information will be linked to a study code. The study code will be used to identify the subject and research images. Research images may be used in scientific presentations, but all images will be de-identified. The data will be stored on the Principal Investigator's (PI or co-PI) password protected computer in her locked office. The code will be stored separate from the data on the PI's computer in her locked office.

9.0 DATA AND SAFETY MONITORING

Safety monitoring of research protocols investigating novel PET tracers utilize a combination of tools. These are framed to provide adequate clinical trial oversight ensuring that the research team is complying with the conditions of IRB approval and FDA requirements. The tools enable frequent evaluations of study-related activities, identification of deviations or noncompliance with the conduct of the study or protocol, review of adverse events tracking and reporting, and review of drug production and accountability.

These tools include:

- Study visit checklists – Research Coordinators use study visit checklists to ensure that all study required procedures and processes are met before, during and after the subject has been seen. Items listed on these checklists include informed consent obtained, consent questions answered (and by whom), vitals recorded, medications reviewed, etc. These checklists are reviewed by the study coordinator and are signed off for each study subject by the coordinator monitoring the study to ensure that protocols are followed accurately and include limited variance from protocol.

- Safety monitoring – The safety monitoring of subjects enrolled in the research includes both internal and external safety checks governing the administration of ^{18}F-DCFPyL. Internal monitoring is performed by co-investigators and external monitoring by a team of four physicians with the appropriate broad clinical and technical. The internal monitoring plan includes a brief review of each subject visit after it occurs. In this regard, the study team will discuss data quality, ^{18}F-DCFPyL production and administration, and subject compliance and tolerance of research procedures.

  The external monitoring plan will be coordinated by the Department of Radiology Medical Imaging Research Support (MIRS) administrative team. MIRS is a working group that provides investigators with the support needed to conduct clinical research that uses imaging as an outcome measure. Members include a core administrative group (physicians, scientists, imaging modality managers, regulatory and administrative support) and ad hoc members recruited when their expertise is required for the review/monitoring of a research project. MIRS administrative team in addition to a 4-person committee of physicians/scientists will meet annually with representatives from the research team, to discuss research progress, AE/SAE reports, as well as other data reports compiled by the study team. If an AE/SAE occurs, an ad hoc committee meeting will be organized to discuss whether the event is considered serious, whether it can be attributed to research procedures, whether it constitutes non-compliance on the part of the study team, and the plan for resolution and a future remediation plan.

  As part of this plan, the monitor(s) will verify that:

  - The rights and well-being of human subjects are protected
  - Reported study data are accurate, complete, and verifiable from source documents
  - The conduct of the study is in compliance with the currently approved protocol, GCPs, applicable regulatory requirements, and guidelines for clinical research studies at the University of Wisconsin-Madison and its affiliates

10.0 STATISTICAL CONSDERATIONS

As noted in section 3.0, the primary objective is to evaluate response to anti-angiogenesis therapy using PSMA-based PET. Other endpoints such as qualitative objective response based on RECIST 1.1 and quantitative CT-based tumor size and SUV parameters will also be evaluated. The secondary objectives are 1) to measure therapy response of patient-derived uVESSEL models using multiple endpoints such as vessel sprouting, PSMA expression and cell death and 2) to evaluate the predictive power and validate the uVESSEL model for clinical outcomes.

10.1 Sample size justification

A total of 15 patients will be recruited into this clinical study. This is based on the feasibility and availability of suitable patients over the funding period with approximately five patients per year. With 15 patients, the width of a 95% confidence interval for the probability of qualitative response based on PSMA-PET and objective response based on RECIST 1.1 will be less than 0.253 and the mean of quantitative endpoints such as CT-based tumor size and SUV parameters will have standard error that is 0.258 times the sample standard deviation.

10.2 Statistical analysis

Statistical analysis will by necessity (n=15) and nature (predictive modeling of response using patient-derived ex-vivo uVESSEL responses as predictors, among others) be exploratory and descriptive including qualitative comparison between responses based on PSMA-PET and RECIST 1.1 without a formal paired test of statistical significance.

Measures of patient response to anti-angiogenics via clinical endpoint such as response based on PSMA-PET (primary endpoint) and objective response based on RECIST 1.1 and histopathological endpoints (tumor vascular density, immunohistochemical staining for PSMA and neovascularization (CD30, CD34)) will be summarized with descriptive statistics (response rate and confidence interval), and changes in these endpoints, PSMA staining from baseline to end of treatment and follow-up) will also be summarized with descriptive statistics. Measures of tumor neovascularization via PSMA-based PET/CT imaging will be summarized with descriptive statistics over time and analyzed using generalized linear mixed-effects models for longitudinal measures. Qualitative ^{18}F-DCFPyL PSMA PET/CT imaging will be recorded in a three-point scale, and statistical analysis will be performed with the equivocal score of 1 considered as positive or negative. Histopathological endpoints will be qualitatively graded by our study pathologist on a zero to four scale with four being the highest level of immunohistochemical staining or neovascularization.

Partial responses are likely from conventional clinical imaging in mRCC patients. Standard imaging techniques define responses to therapy based on size changes of target lesions using RECIST1.1. Metastatic tumors may respond to anti-angiogenic treatment but not change substantially in size (unlike cytotoxic therapies for which RECIST was designed). To address this limitation in current imaging, we combine standard imaging with functional imaging protocols to evaluate whether angiogenesis decreases in individual metastatic lesions regardless of changes in tumor size. PSMA PET signal is used as a surrogate for changes in tumor neovasculature. These functional imaging findings will be

confirmed pathologically by evaluating angiogenesis/ PSMA expression in tumor samples and patient models. Using this approach, we will evaluate functional anti-angiogenesis responses to therapies.

For the uVESSEL models (secondary objective 1), generalized linear mixed-effects models for longitudinal measures taken four times will be used for various endpoints with treatment (control plus four anti-angiogenics), dose (three levels each replicated three times), and plate (primary vs metastatic crossed by normal vs tumor) as the main factors. This will allow evaluation of the effects of treatment and dose and the differences between primary and metastatic samples and between normal and tumor vessels. We will also include these two models as the main factor in a combined generalized linear mixed-effects model, which will allow direct comparison of the biomimetic and the organotypic models.

Predictive modeling (secondary objective 2) will thus include the actual tumor size change (in a typical waterfall plot) and the dichotomy of disease control vs no disease control in the predictive model for tumor response in addition to the dichotomy of responders vs. non-responders in functional studies.

Due to limited sample size (n=15) and because not all patients are treated with the same anti-angiogenic therapy, further reducing the sample size for each anti-angiogenic therapy, one cannot expect the predictive analysis to give a clear discrimination between responders and non-responders to anti-angiogenic therapies. As an alternative, we will also use clustering analysis based on k-means or Gaussian mixture models which are less sensitive to small sample sizes as in Pak (2015).

Using the clinical, pathological and imaging endpoints as dependent variables, we will develop predictive models using responses to the uVESSEL model as independent predictor variables. More specifically, we will use the objective response as a dichotomous endpoint as a dependent variable in a logistic regression model with response to the uVESSEL model as an independent predictor. This approach is equivalent to linear discriminant analysis. Although we expect about 70% of eligible patients will receive pazopanib and the rest sunitinib, we can still use the objective response as the dependent variable in logistic regression as both pazopanib and sunitinib are expected to give a similar response rate of around 30% in this patient population. In addition each patient sample will be subject to both agents in the uVESSEL model and provide response to be used as predictors in logistic regression modeling.

10.3 Feasibility

In 2016, there were 184 new patients with renal cell carcinoma seen at UWHC, with between 33-50% of patients having locally advanced or metastatic tumors. There are no competing studies for the current project, and we anticipate no issues with planned enrollment.

11.0 DRUG FORMULATION AND PROCUREMENT

Investigation Product

The investigational product will be obtained from the University of Wisconsin Radiopharmaceutical Production Facility (RPF). The RPF is located in the Wisconsin Institute for Medical Research Building. The FDA IND for this protocol will be cross-filed with the current Johns Hopkins University IND for 18F-DCFPyL, IND # 121064 to reference the Pharmacology and Toxicology, dosimetry, and Previous Human Experience sections of that IND.

Common Name: DCFPYL

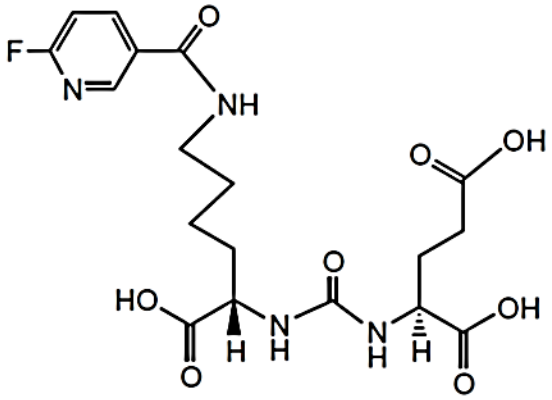
Chemical Names: 2-(3-{1-Carboxy-5-[(6-fluoro-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid

Characteristics: hygroscopic white powder

Chemical Formula: $C_{18}H_{23}FN_4O_8$

C.A.S. Number: 1423758-00-2

Structure:



Molecular Weight: 442.40

Solubility: Soluble in DMSO, methanol, 1:1 acetonitrile:water

^{18}F-DCFPyL is eluted with 1 mL of ethanol followed by 10 mL of 0.9% sodium chloride, via a sterilizing 0.22µ filter into a sterile vial containing 4 mL 0.9% sodium chloride. Test radiopharmaceutical will be administered intravenously via slow I.V. push. The maximum mass dose of the ligand corresponding to the 9 mCi dose of ^{18}F-DCFPyL will be less than 3.98 µg per administration, although the actual dose will be significantly less depending on the specific activity achieved during each radiosynthesis run. The lowest limit for specific-activity (SA) we will use at the time of injection is 1000 mCi/µmole, although the validation and phase I study radiosynthesis SA was significantly higher than 1000 mCi/µmole.

Preclinical Toxicology

A toxicity report "*14-Day Study to Determine Toxicity of DCFPyL from a Single IV Dose in Sprague Dawley Rats*" was prepared by SoBran Inc. (Study No. SB-MP-001). The following summary is taken from that report.

The purpose of this study was to evaluate the toxicity of DCFPyL three and fifteen days following a single intravenous dose in rats. This study consisted of two test article treatment groups of ten male and female Sprague-Dawley rats per group dosed with DCFPyL at 0.1 and 0.5 mg/kg. An additional group of ten males and ten females received the vehicle, 5% Dextrose, and served as the control. All rats received a dose volume of 5 mL/kg. The rats were dosed intravenously once on Study Day 1. Five male and five female rats from each group were bled on Study Day 3 and the remaining rats

were bled on Study Day 15. All animals were euthanized and necropsied following blood collection. Parameters evaluated for test article effect included survival, clinical observations, body weight, body weight gain, clinical pathology, gross pathology, organ weights, and microscopic pathology.

All rats survived to the scheduled termination and remained bright, alert and responsive during the study. No abnormal findings were indicated during cageside or hands-on observations. One female rat treated with the vehicle control and one female rat treated with 0.1 mg/kg DCFPyL lost weight between Days 8 and 15. All rats treated with 5% Dextrose or DCFPyL gained weight during the course of the study. There were no treatment related changes seen in the hematology, coagulation, or clinical chemistry data. Organ weights showed some variance but microscopic findings in the Day 3 and Day 15 rats were considered incidental and not directly related to the test article. Under the conditions of this study, there were no treatment related findings in Sprague Dawley rats three or fifteen days after a single intravenous dose of DCFPyL at 0.1 mg/kg and 0.5 mg/kg.

Organ	30 min	60 min	120 min	240
Blood	1.53 ± 0.19	0.24 ± 0.05	0.43 ± 0.37	0.0
Heart	0.68 ± 0.07	0.20 ± 0.11	0.06 ± 0.01	0.0
Lung	1.91 ± 0.47	0.55 ± 0.17	0.18 ± 0.02	0.0
Liver	3.88 ± 0.74	2.87 ± 0.92	2.14 ± 0.11	1.8
Stomach	1.50 ± 1.12	0.35 ± 0.34	0.08 ± 0.03	0.0
Pancreas	1.02 ± 0.53	0.26 ± 0.13	0.08 ± 0.00	0.0
Spleen	7.59 ± 3.56	2.70 ± 1.28	0.69 ± 0.11	0.2
Kidney	74.1 ± 6.6	42.3 ± 19.0	15.7 ± 3.3	7.4
Muscle	0.39 ± 0.05	0.61 ± 0.92	0.04 ± 0.00	0.0
Bone	0.82 ± 0.16	0.42 ± 0.15	0.33 ± 0.08	0.4
sm. Intest	0.79 ± 0.11	0.31 ± 0.12	0.11 ± 0.07	0.0
lrg. Intest	0.73 ± 0.04	0.40 ± 0.17	0.12 ± 0.05	0.0
Bladder (empty)	18.6 ± 18.1	9.88 ± 4.92	6.44 ± 4.42	1.5
PSMA+ PIP	46.7 ± 5.8	44.2 ± 9.7	39.4 ± 5.4	36.
PSMA⁻flu	1.17 ± 0.41	0.36 ± 0.14	0.11 ± 0.02	0.0
PIP/flu	40	123	358	122

[a]Values are in% ID/g SD; $n = 4$.

^{18}F-DCFPyL was assessed for its ex-vivo pharmacokinetics in non-obese diabetic severe-combined immunodeficient (NOD-SCID) mice bearing both PSMA positive PC3-PIP and PSMA negative PC3-flu xenografts. Table below shows the percent injected dose per gram of tissue (%ID/g) of ^{18}F-DCFPyL activity in selected organs.

^{18}F-DCFPyL PSMA-dependent uptake within PSMA positive PC3 PIP xenografts, reaching a value of 46.7 ± 5.8%ID/g at 30 minutes post-injection (p.i.), which decreased by only about 10% over the ensuing 4 hours. At 60 minutes p.i. the kidney, liver, and spleen displayed the highest uptake. By that time, the urinary bladder also showed relatively high uptake. However, that uptake includes excretion at all-time points. Rapid clearance from the kidneys was shown, decreasing from 74.1 ± 6.6%ID/g at 30 minutes to 7.4 ± 0.9%ID/g at 4 hours. The relatively high values noted in kidney are partially due to high expression of PSMA within proximal renal tubules. The ratio of uptake within PSMA positive PIP to PSMA negative flu tumors ranged from 40:1 to more than 1,000:1 over the 4-hour time period of the study. A possible explanation for that increased tumor uptake of radiochemical over time could be due to ligand-mediated PSMA internalization within tumor cells. Less retention in

kidney relative to tumor over time could be due to a lower degree of internalization in this (normal) tissue and/or different metabolism of ^{18}F-DCFPyL, which does not promote retention of radiochemical in kidney. Relatively low bone uptake (<1% ID/g at all-time points) suggests little metabolic defluorination of ^{18}F-DCFPyL. The total effective dose for 333 MBq (9 mCi) of ^{18}F-DCFPyL was calculated to be 5.93 mGy (0.593 rem) based on animal models.

Initial Phase I Study Adverse Events:
An initial phase I study of the biodistribution and dosimetry of ^{18}F-DCFPyL found that in nine patients there were no severe adverse events. One patient reported two adverse events that were classified as unlikely to be attributable to the radiotracer (mild headache and mild nose bleed, both of which resolved without treatment and were considered Grade I by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0). Another participant experienced a decrease in platelet count on routine assessment during the post-imaging follow up which, at the time of publication, had not resolved but which was attributed to the participant starting treatment for prostate cancer. This was also a NCI CTCAE Grade I adverse event. There were no radiotracer-related adverse events with monitored heart rate or blood pressure.

Human dosimetry: 18F-DCFPyL human dosimetry reported in Risks/Benefits section.

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