LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT #4

LCCC 1930: Quantitative PET-MRI Imaging Correlated with Transcriptome Analysis for Noninvasive Characterization of Renal Cell Carcinomas

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
___ Scientific changes
___ Therapy changes
_x__ Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY:

The changes included in this amendment represent a better understanding of the patient population and clinical practices of UNC providers. Numerous patients have been evaluated since the beginning of the trial who have a primary renal mass smaller than 7 cm but with clear evidence of advanced disease. Additionally, as it is standard of care for patients with distant disease to receive a cytoreductive nephrectomy, inclusion of patients from this sub-set of the population could strengthen our understanding of the heterogeneity of clearly more aggressive ccRCC tumors.

List of updates to the protocol:

1. The radiological evaluation of tumor size was changed from ≥ 7 cm to ≥ 4 cm as inclusion criteria in the following sections: 1.3 and 3.1.3.

2. The number of samples to be collected was changed in section 1.1 to correspond with the study design as described in section 7.1.

3. The exclusion criterion 3.2.7 "Evidence of distant disease on physical exam or initial imaging" has been removed. It is standard practice for patients with distant ccRCC to undergo a cytoreductive nephrectomy and inclusion of these patients could further inform risk-stratification of patients with ccRCC.

4. The tumor size listed in 7.1 was changed from ≥ 7 cm to ≥ 4 cm.

5. The exclusion criterion 3.2.1 was updated to clarify patients suffering from claustrophobia severe enough to require sedation will be excluded from the study.

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PROTOCOL AMENDMENT #3

LCCC 1930: Quantitative PET-MRI Imaging Correlated with Transcriptome Analysis for Noninvasive Characterization of Renal Cell Carcinomas

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
___ Scientific changes
___ Therapy changes
_x__ Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY:

The size and BMI criteria are unnecessary for the successful execution of the protocol and have led to the exclusion of many potential candidates who otherwise may have been good study subjects.

List of updates to the protocol:

1. Removed size restrictions from section 3.1 Inclusion Criteria.
2. Removed BMI limit from section 3.2 Exclusion Criteria.

THE ATTACHED VERSION DATED April 21, 2021 INCORPORATES THE ABOVE REVISIONS
ATTACH TO THE FRONT OF EVERY COPY OF PROTOCOL

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT #2

LCCC 1930: Quantitative PET-MRI Imaging Correlated with Transcriptome Analysis for Noninvasive Characterization of Renal Cell Carcinomas

AMENDMENT INCORPORATES (check all that apply):

_x_ Editorial, administrative changes
___ Scientific changes
___ Therapy changes
_x__ Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY:

Clarification of study procedures drives the changes of this amendment. The Lineberger Comprehensive Cancer Center (LCCC) Office of Clinical and Translational Research (OCTR) is now supporting portions of this work. Many of the changes in language in this amendment accommodate the standard procedures for OCTR.

List of updates to protocol:

1. OCTR compliance statement added to section 1.4
2. Secondary objective moved to exploratory section
3. Eligibility criteria 3.1.2 modified to incorporate signing of both adult consent and HIPAA authorization
4. Eligibility criteria 3.1.4 modified to indicate surgery is standard of care, and not planned per research protocol
5. Eligibility criteria 3.1.7 modified to indicate nephrectomies must be done in Chapel Hill, as there is currently no mechanisms to collect mulitfocal tissue from other institutions.
6. Eligibility criteria 3.1.9 regarding pregnancy tests modified to match IRB application. This modification requires pregnancy testing on the day of Imaging, rather than within 7 days prior to imaging.
7. Exclusion criteria 3.2.1 added the need for lorazepam to tolerate MRI.
8. Exclusion criteria 3.2.2 edited for clarification that gadolinium contrast hypersensitivity should be documented
9. Exclusion criteria 3.2.8 and 3.2.11 edited to use standard OCTR language
10. Study schema 4.1 edited for clarity, schema and study plan has not changed
11. Section 4.2 regarding patient identification and consent updated to include standard language
12. Section 4.4 edited to clarify that dosing is measured and recorded, but cannot be controlled precisely.
13. The time and events table in section 5.0 updated for clarity, time and events have not changed.
14. Section 7.4 Data management was updated to describe how patient information would be maintained
15. Sections 8.2, 8.3, 8.4, 8.5.2 were updated to standard contain language for standard OCTR and Lineberger procedures

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UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1930: Quantitative PET-MRI Imaging Correlated with Transcriptome Analysis for Noninvasive Characterization of Renal Cell Carcinomas

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Version Date: August 06, 2021

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1930: Quantitative PET-MRI Imaging Correlated with Transcriptome Analysis for Noninvasive Characterization of Renal Cell Carcinomas

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Signature Page

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 U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: David S. Lalush, PhD

PI Signature: _____

Date:_____

Version Date: August 06, 2021

TABLE OF CONTENTS

1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

The objective of this study is to evaluate the utility of simultaneous positron emission tomography (PET) and magnetic resonance imaging (MRI) in characterizing the molecular subtypes of clear cell renal cell carcinomas (ccRCC) to potentially inform prognosis and treatment decisions. Previous results on a small number of subjects at UNC demonstrated a relationship between PET-MRI imaging characteristics and tumor subtype composition as determined by transcriptome analysis of surgical core samples in ccRCC. ccRCC has been classified into two molecular subtypes, ccA and ccB, on the basis of expression of a group of 34 genes; the two subtypes have been shown to have clear differences in prognosis. These tumors are notoriously heterogeneous; a single tumor can contain both subtypes, and full tumor molecular characterization is infeasible. Imaging, however, offers a snapshot of the whole tumor. If imaging characteristics prove to be predictive of subtype composition, early prognostic information would be available and different approaches to treatment may be enabled.

In this project, we will image 17 ccRCC subjects on simultaneous PET-MRI and quantify the metabolically-active fraction of the tumor from images. Up to ten core samples will be taken from each tumor post-surgery and classified as ccA or ccB subtype using transcriptome analysis. The imaging-based measures will be correlated with the fraction of tumor cores classified as ccB. We hypothesize that the metabolically-active tumor volume fraction derived from simultaneous PET-MRI imaging is correlated with the fraction of tumor cores classified as ccB subtype from transcriptome analysis.

This study is designed to provide preliminary information on the relationship between imaging metrics and tumor subtype composition in 17 ccRCC subjects. The primary objective is to evaluate the capabilities of MRI/PET in prediction of ccB burden.

Information from this study may be used to estimate effect size for power calculation and sample size considerations for possible future studies in the use of combined PET-MR to be used for prognostic information in ccRCC cases and potentially to enable risk-stratification that is not currently possible.

1.2 Background

Simultaneous PET/MR imaging [1]–[7] offers exciting opportunities to visualize and quantify soft-tissue tumors [2]. MRI offers superb soft-tissue contrast for anatomical information as well as a flexible suite of other techniques providing functional and physiological information. PET offers sensitive molecular imaging via radioactive tracers and is widely used for assessment of tumor glucose metabolism. Together, the two modalities provide complementary, synergistic information. Because of MRI's superior soft-tissue contrast, it is considered a much better anatomical guide

for PET quantitative analyses for tumors in soft tissue regions as compared to standard-of-care PET-CT. Simultaneous PET-MR also provides inherently-aligned PET and MR, efficient simultaneous acquisition, and the opportunity for new approaches to PET quantitative analysis guided by detailed MR images.

Nonmetastatic clear cell renal cell carcinomas are generally treated with nephrectomy, although research has subclassified them into two [8] or as many as four [9] subtypes based on gene expression; the subtypes have been shown to have differing prognoses and progression [8]–[11]. We focus primarily on the two subtypes ccA and ccB [8] and ask whether these two exhibit distinct PET-MRI imaging characteristics that can be used to subtype them noninvasively. An eight-subject pilot study conducted on PET-MRI at UNC demonstrated that quantitative PET-MRI imaging, using nonstandard measures incorporating the entire tumor, was strongly correlated with presence or absence of ccB patterns in the tumors [12]. Transcriptome analysis results from the study justified the import of PET in noting that many of the upregulated genes associated with ccB were involved in glucose transport and metabolism, the mediators of 18-FDG uptake. Thus, there is a strong biologic rationale as to why PET-MRI could predict ccB burden, but the small number of subjects in that study limits confidence. Also, the fact that these tumors are highly heterogeneous suggests that imaging alone provides an opportunity for early characterization of ccB burden across the entire tumor where gene expression profiling would be invasive and costly. Also, with the excellent soft-tissue contrast of MRI, the system has the potential for greater anatomical detail to guide interpretation of FDG activity. UNC has one of the few PET-MR scanners in the country, making it one of few centers in the US capable of conducting this study.

1.3 Purpose and Rationale

This is a prospective study of the use of combined PET-MR [1-7] for prediction of tumor subtype composition in ccRCC. Additional data analysis using the prior study from UNC suggests a strong correlation between the fraction of the tumor exhibiting moderate to high FDG uptake and the number of cores found to be ccB type. These results guide the hypothesis of this study: the metabolically-active tumor volume fraction derived from simultaneous PET-MRI imaging of clear-cell renal cell carcinomas is correlated with the fraction of tumor cores classified as ccB subtype from transcriptome analysis. If so, in the future, application of PET-MRI may offer prognostic information and opportunities for risk stratification.

We propose that the results of a single PET-MRI study prior to surgery will predict ccA/ccB content of the tumor. Patients will have clinically-suspected ccRCC based on prior imaging, with tumor size $\geq$ 4cm. Subjects must have a planned nephrectomy schedule that will allow PET-MRI imaging within 4 weeks prior to surgery. Once patient identification occurs, the patient will be assessed for the study via the inclusion and exclusion criteria. Among other criteria, patients must have no contraindications against 18F-FDG PET nor MRI with gadolinium contrast. After recruitment by the study coordinator and informed consent, the patient will be admitted to the study and scheduled for imaging.

Patients in our study will receive their PET/MRI scan on the Biograph mMR combined PET-MRI scanner at the Biomedical Research Imaging Center. The PET-MRI scans will be evaluated retrospectively and will not be used in the determination of treatment.

Quantitative measures from PET and MRI will be computed: Quantitative measures from PET will be computed, including tumor maximum SUV, tumor mean SUV, and fraction of tumor volume over threshold SUV 2.0 (FTVOT2), as well as Metabolic Tumor Volume (MTV) and Total Lesion Glycolysis (TLG).

Quantitative measures from MRI will be computed including tumor volume, apparent diffusion coefficient, dynamic contrast enhancement, and tumor water content. All quantitative measures will be compiled into a complete image-based characterization of each tumor.

Patients will undergo nephrectomy within 4 weeks of imaging. Up to ten samples will be taken from each tumor, spatially distributed, and stored according the lab manual. Non-ccRCC histologies will be excluded from further analysis. RNA will be extracted and each sample will be classified as ccA or ccB using the ClearCode34 Nanostring classifier as previously described using a 34-gene custom Nanostring probe (NanoString Technologies, Seattle, WA).[13] Each sample will then be categorized as ccB or ccA or equivocal. The fraction of samples classified as ccB will be used in further analysis.

1.4 Compliance Statement

This study will be conducted in full accordance to all applicable University of North Carolina (UNC) Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. Any episode of non-compliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent, and will report unexpected problems in accordance with The UNC IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2.0 STUDY OBJECTIVES/AIMS AND ENDPOINTS

2.1 Primary objectives

2.1.1 To correlate quantitative metrics from imaging characterizing the tumor volume fraction associated with medium-to-high 18-FDG-PET uptake with the fraction of ccB-positive cores based on transcript analysis

2.3 Exploratory Objectives

2.3.1 [redacted]

2.3.2 [redacted]

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

Subject must meet all of the inclusion criteria to participate in this study:

3.1.1 Age ≥ 18 years of age (no upper age limit)

3.1.2 Has voluntarily provided signed informed consent to participate and HIPAA authorization for release of personal health information.

3.1.3 Must have clinically-suspected ccRCC based on prior imaging with tumor size ≥ 4 cm.

3.1.4 Planned to have standard of care nephrectomy with a schedule that can accommodate a MR-FDG-PET scan within 4 weeks prior to surgery.

3.1.5 Must be able to understand and comply with study procedures for the entire length of the study.

3.1.6 Must receive their nephrectomy at UNC Chapel Hill Hospitals.

3.1.7 If patient has a history of prior malignancy, including melanoma, patient must be cancer-free for three or more years. Non-melanoma skin cancers will be included even if not cancer-free for three years.

3.1.8 Women of childbearing potential must have a negative urine pregnancy test performed the day of and prior to PET/MRI imaging.

3.1.9 Willing to undergo finger-stick blood glucose tests.

3.2 Exclusion Criteria

Any subject meeting any of the following exclusion criteria will be ineligible for study participation:

3.2.1 Inability to tolerate MRI (e.g., inability to lie flat for >1 hour or claustrophobia

requiring lorazepam to tolerate MRI),

3.2.2 Documented hypersensitivity to gadolinium contrast agent

3.2.3 Presence of pacemaker, intracranial aneurysm clip, bladder stimulator, cochlear implant or metal near eyes or near pelvis that would create excessive imaging artifact

3.2.4 Failure of two blood glucose tests (blood glucose level greater than 200 mg/dL) on day of first scheduled scan visit and on rescheduled day.

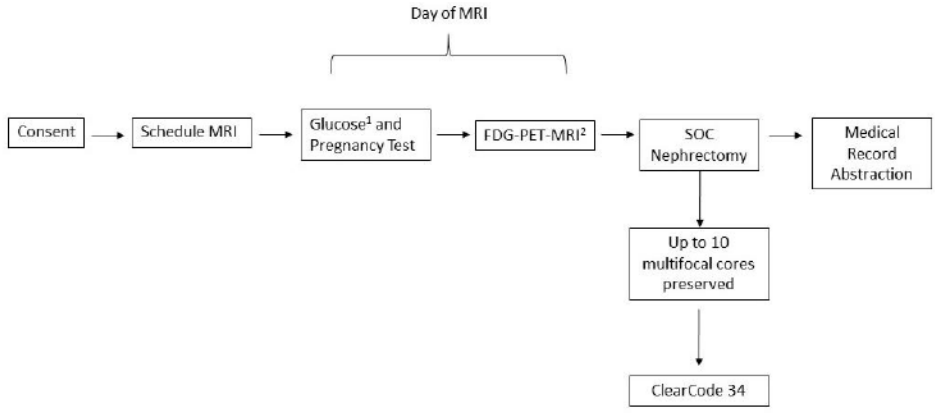
3.2.5 Patient receiving neoadjuvant therapy for RCC

3.2.6 Unwilling or unable to complete informed consent

3.2.7 Incarcerated patients or patients with medical power of attorney at time of enrollment

4.0 STUDY PLAN

4.1 Schema



[1] If glucose test is not passed (>200mg/dL) first time, subject can wait 30 minutes and repeat the test. If failed again, the subject can reschedule MRI and repeat glucose and pregnancy test. If failed twice on second try, the subject will be rescheduled for another day or excluded from the study.

[2]Subject will be given pre-MRI instructions. If both creatinine is <1.8mg/DL and glucose test is <200mg/dL, then subject can undergo FDG and Gadolinium contrasting portions of protocol. If only glucose test is passed, then only the FDG imaging will be done.

4.2 Patient Identification and Consent

All patients with an appointment for a renal mass at UNC Chapel Hill Hospital may

be approached in person for consent. In a private clinic room, study staff will review consent documents with potential participants and answer any questions. If a patient desires additional time or wishes to delay enrollment in the study, he/she will be given a copy of the consent document and informed about how to ask questions or enroll at a later date. Enrollment and informed consent will be facilitated by the study coordinator. If the subject chooses to participate in the study, he or she signs the consent and is given a copy for his/her own records.

If at any point in this process the patient declines, fails to meet eligibility criteria, or is otherwise unable to fulfill the requirements of the research, he or she is excluded from further participation. Patients will be provided assurance that declining participation will not affect their clinical care.

4.3 Blood Draw for Creatinine

If subjects do not have a serum creatinine value within 30 days prior to a scheduled PET-MRI scan, they will be required to have a blood draw at UNC Hospitals for creatinine before their PET-MRI scan visit. This is the normal procedure prior to nephrectomy, and so these blood values can also be used for PET-MRI scans. . Serum creatinine must be less than 1.8mg/dL in order to proceed with the Dynamic Contrast Enhancement MRI part of the scan. If the subject does not meet that level, the subject will be imaged with the other non-contrast sequences and with PET since the non-contrast images will still meet the primary objective of the study.

4.4 FDG-PET-MRI

All patients will undergo a MRI scan with simultaneous acquisition of [^{18}F] Fludeoxyglucose Positron Emission Tomography (FDG F18- PET). 18F-FDG is a positron-emitting radiopharmaceutical used for diagnostic purposes. It is a glucose analog that concentrates in cells relying upon glucose as an energy source or in cells whose reliance on glucose increases under pathophysiological conditions. Each participant will receive approximately 12 mCi 18F-FDG, the exact dose administered to each participant will be measured and recorded in the study records

Subjects will have a finger-stick blood glucose test upon reporting to the imaging center; blood glucose must be below 200 mg/dL in order to proceed with the FDG injection. If blood glucose exceeds this level, a second finger-stick test may be performed after 30 minutes, and if the second test is below the threshold, the FDG injection may proceed. Failing the second test will result in the subject being rescheduled for another day or excluded from the study.

Injection of gadolinium contrast agent (if the serum creatinine test is passed: serum creatinine < 1.8 mg/dL) will be performed in the usual manner and images simultaneously obtained with MRI. If the serum creatinine is above the threshold, the subject will not receive gadolinium contrast, but the remainder of the scanning protocol will proceed.

Patients will have fasted for at least 6 hours before intravenous injection of FDG. To minimize radiation-absorbed dose to the bladder, patients should drink at least an 8 ounce glass of water prior to drug administration. Whenever possible, patients should take the following precautions for 12 hours after injection: used toilets should be flushed several times after each use, and hands should be washed thoroughly. If blood, urine or feces soil clothing, the clothing should be washed separately.

Each patient will be imaged at a single visit. Patients will be scheduled for PET-MRI imaging at Marsico Hall and will receive imaging-day instructions from the study coordinator. The study coordinator will meet the patient at the imaging facility and escort them to the imaging suite. Women of childbearing potential will undergo urine pregnancy test the day of and prior to the PET/MRI.

Patients will be paid $50 each upon completion of the imaging visit as compensation for their time.

4.5 Duration of Study Intervention

The study intervention is complete once the patient receives their PET-MRI scan. The patient may be withdrawn from the study prior to this point if any of the following apply:

- Inter-current illness prevents completion of imaging studies
- Unacceptable adverse event(s) prevents completion of imaging studies
- Patient decides to withdraw from the study, **OR**
- General or specific changes in the patient's condition render the patient unacceptable for completion of study in the judgment of the investigator.

After PET-MRI, patients will undergo nephrectomy as planned and per standard of care and tumors will be sampled as above (up to 10 cores will be taken from each tumor post-surgery and classified as ccA or ccB subtype using transcriptome analysis).

4.6 Duration of Follow Up

Patients will be followed up via review of their medical records through disease recurrence or survival for up to 5 years.

4.7 Removal of Patients from Protocol

Patients may be removed if they experience unanticipated claustrophobia causing intolerance to the MR. If patients require lorazepam in order to tolerate the MR, they will be withdrawn from study participation.

4.8 Abstraction of Medical Records

Medical records will be reviewed and data extracted for clinical outcomes. Pathologic stage, as well as any information on recurrence and survival.

Information collected may help to establish preliminary data for future studies.

5.0 TIME AND EVENTS TABLE

	Pre-Study	Imaging	Surgery	Long-term follow-up[1]
Screening	X			
Informed Consent	X			
Pregnancy test[2]		X		
Creatinine blood draw	X[3]			
PET-MRI		X		
Glucose Test		X		
Surgery			X	
Collection of Surgical Tissue			X	
Abstraction of Medical Records				X

[1]Long-term follow-up will be restricted to abstraction of medical records for any data on recurrence and/or survival for up to five years post treatment.
[2]If clinically applicable women of childbearing potential will undergo urine pregnancy test the day of and prior to PET-MRI imaging
[3]Creatinine should be drawn within 30 days of PET-MRI imaging

6.0 EXPECTED RISKS/UNANTICIPATED PROBLEMS

6.1 Assessment of Safety

In general, any patient enrolled on this protocol and who undergoes any portion of the PET-MRI study (including gadolinium administration) will be evaluable for adverse events.

6.2 Expected Risks

6.2.1 Risks of PET/MRI

Emotional Distress

Emotional distress is possible during MRI. Technologists will ask subjects, before injection, if they are claustrophobic. Also, technologists will do their best to help comfort any subject who is claustrophobic but chooses to continue. Technologists will hand subjects a squeeze ball alarm and instruct them to use it in case of any discomfort. The technologist will also inform the subject that she is free to stop at any time, for any reason.

Radiation

Radiation: The PET/MRI scans will expose study participants to controlled amounts of limited radiation. The total dose of radiation from these tests is not anticipated to cause any adverse effects of any significance over that which they may experience over their standard of care diagnostic imaging and subsequent therapies. Patients enrolled in this pilot study will receive an estimated dose of radiation as specified by the Radiation Safety Committee. The amount of risk to this estimated dose will be equated to the annual radiation exposure limit for radiation workers in the informed consent. This radiation exposure involves a small risk and is necessary to obtain the information desired.

This research study involves exposure to radiation from radiotracer (approximately 12 milliCuries 18F-FDG) used for PET/MR scan. The radiation dose subjects will receive in this study is 1.3 rem. For comparison, a person in the United States receives a radiation exposure of 0.3 rem per year from natural background sources. The radiation dose that subjects will receive in this study is equivalent to the radiation exposure that everyone receives in 4.3 years from natural background radiation for participants completing the PET scan. For comparison, the people who work with radiation (radiation workers) are allowed to receive a radiation dose of 5 rem per year. The amount of radiation exposure received in this study is equal to 26 % of the annual radiation exposure limit for radiation workers. This radiation exposure involves only a small risk and is necessary to obtain the research information desired. The radiation exposure described here is what subjects will get from this research study only. It does not include any exposure subjects may have received or will receive from other tests outside of this study that are a part of their medical care.

FDG

Information about FDG F18 was obtained from the Prescribing Information (August 5 2004); http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ . According to this document , reviews of the oncology literature did not reveal reported adverse reactions when using 18F-FDG as a diagnostic in conjunction with PET. In a subset (n=42) of a safety database of epilepsy patients (n=374), 4 patients had transient hypotension, 6 had hypo- or hyperglycemia and 3 had transient increases in alkaline phosphatase.

Gadolinium

An extremely rare disease called Nephrogenic Systemic Fibrosis (NSF) is associated with the use of gadolinium contrast agents in patients with chronic severe renal insufficiency or renal dysfunction due to hepato-renal syndrome or in the peri-operative liver transplantation period. Exclusion criteria for this study are in compliance with the Food and Drug Administration's advisory statements.

As part of the MRI procedure subjects may receive a dye called gadolinium. Gadolinium makes it easier to see details on the MRI pictures. If subjects have any problems with their kidneys, they may be at risk for a condition called Nephrogenic

Systemic Fibrosis (NSF) or Nephrogenic Fibrosing Dermopathy. NSF has been reported to occur between 2 days and 18 months following injection of gadolinium. There is no known treatment for NSF. Some people have even died from this. Signs and symptoms of NSF may include: burning, swelling, hardening or tightening of the skin, blood vessels and internal organs (heart, lungs, liver; yellow spots on the white part of the eyes; joint swelling and stiffness; pain in the hip bones or ribs; muscle weakness.

Subjects' study doctor will check how well their kidneys work before they are given gadolinium. Depending on how well their kidneys work, they may not be given gadolinium at all. NSF has not been reported in people with normal kidneys.

Small amounts of gadolinium can stay in the body including the brain, bones, skin and other parts of the body for a long time (several months to years) in people with normal kidney function. People who get many doses of gadolinium medicines, women who are pregnant and young children may be at increased risk from gadolinium staying in the body. So far, studies have not found harmful effects of gadolinium in patients with normal kidneys. Rarely, patients have reported pains, tiredness, and skin, muscle or bone ailments for a long time, but these symptoms have not been directly linked to gadolinium.

6.3 Unanticipated Problems

6.3.1 Definition

As defined by UNC's IRB, unanticipated problems involving risks to study subjects refers to any incident, experience, or outcome that:

- Is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Is related or possibly related to a subject's participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

6.3.2 Reporting

Any unanticipated problem that occurs during the conduct of this study and that meets **at least** the first two criteria listed in 6.3.1 must be reported to the UNC IRB using the IRB's web-based reporting system.

6.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data including the number of patients treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

7.0 STATISTICAL CONSIDERATIONS

7.1 Study Design/Study Endpoints

The purpose of the study is to assess the utility of combined, simultaneous positron emission tomography (PET) and magnetic resonance imaging (MRI), collectively called PET-MR, in predicting the fraction of tumor cores expressing the ccB subtype. The overall design is to image patients with PET-MR prior to surgery and evaluate quantitative metrics from imaging, and to evaluate the correlation of imaging metrics with the results of post-surgery transcriptome analysis. The primary endpoint is to correlate the MRI-guided PET metric fractional tumor volume over threshold SUV > 2.0 (FTVOT2) with the fraction of tumor cores classified as ccB from transcriptome analysis.

Patients will have suspected ccRCC based on imaging with tumor size ≥ 4 cm and for whom surgical treatment is planned. Once patient identification occurs, the patient will be assessed for the study via the inclusion and exclusion criteria mentioned above. The patient will then be approached by a study coordinator and

the study protocol explained and all risks outlined. If the patient provides informed consent the patient will be enrolled. Demographic data such as patient age, gender, comorbidities, and previous treatments, etc. will be collected. Tumor characteristics will be collected. All data collected will be kept on a password protected, secured server, and all physical documentation will be stored in a locked cabinet. All data will be collected by the PI, co-investigators and study coordinators.

Patients will then be scheduled for a pre-surgery PET-MR within four weeks of planned surgery.

For all PET scans, patients will be instructed to fast, and blood glucose level will be measured prior to injection of FDG; if blood glucose exceeds 200 mg/dL after two finger-stick tests, the patient will be rescheduled for another day or excluded from the study. Patients will be injected with approximately 12 mCi 18F-FDG, and then imaged starting 60 minutes post-injection for ten minutes each at one or two bed positions focused on the primary tumor site. MRI sequences to be run will include conventional anatomic T1- and T2-weighted sequences, high-resolution T1 pre- and post-contrast DCE sequences, and the PET-MR attenuation-correction sequence.

The PET-MRs will be evaluated retrospectively by study personnel as to the size of the lesion, the FDG activity as measured by tumor-maximum and tumor-mean standard uptake values (SUVs), additional PET metrics (metabolic tumor volume (MTV) and total lesion glycolysis (TLG)), the presence or absence of invasion to adjacent organs, and the presence or absence of metastatic or multifocal disease. PET images will be evaluated with aligned anatomical MRI for guidance as well as determination of the tumor margins for computation of tumor-mean SUV, and FTVOT2.

The pathological specimens will be evaluated by a UNC pathologist. Again, all data will be stored on a secured, password protected server. Up to ten samples will be taken from each tumor, spatially distributed, and stored according to lab manual. Non-ccRCC histologies will be excluded from further analysis. RNA will be extracted and each sample will be classified as ccA or ccB using the ClearCode34 Nanostring classifier as previously described using a 34-gene custom Nanostring probe (NanoString Technologies, Seattle, WA).[13] Each sample will then be categorized as ccB or ccA or equivocal. If both tumor types appear in the same sample, the classifier will choose the closest match; however, each result will be reviewed and samples that show clear elements of both types in expression patterns will be labeled as equivocal. The fraction of samples classified as clearly ccB will be used in further analysis.

To address the primary endpoint, FTVOT2 will be evaluated and recorded for each subject as will the fraction of samples classified as ccB.

7.2 Sample Size and Accrual

The study will enroll 17 eligible patients that are being treated at UNC Hospitals.

7.3 Data Analysis Plans

For the primary endpoint, each subject's FTVOT2 will be correlated with the primary outcome (fraction of core classified as ccB) and correlation estimated using the non-parametric Spearman correlation test.

Regarding the specific hypothesis testing of PET FTVOT2, with N = 17, we will have approximately 54% power to detect a correlation of rho = 0.5 (and 74% power to detect rho = 0.6), with a Type I error rate of 0.05 and utilizing the Spearman correlation test. This is considered a conservative estimate. Data from the prior study[1] shows a strong Spearman correlation (rho = .896, p = .006) between the fraction of core samples classified as ccB and the PET tumor volume fraction over SUV 2.0 (FTVOT2).

[1] S. A. Brooks et al., "Alternate metabolic programs define regional variation of relevant biological features in renal cell carcinoma progression," *Clinical Cancer Research*, Jan 19, 2016. (PMID: 26787754)

7.4 Data Management

Image datasets will be stored on a secure server in the Biomedical Research Imaging Center with patient identifiers removed. De-identified copies of these may be stored temporarily on password-protected computers or portable hard drives for use in data analysis. Quantitative data extracted from the images and results of data analysis will be maintained on a secure server. All data will be archived on a password-protected computer in the office of the PI and backed up with a RAID system. Datasets will be maintained for at least five years from the close of the study.

All subject information will be coded and stored in a password-protected database, such as REDcap, and accessible by trained study staff only. All specimens will be given another code, and will not be stored with subject identifiers. The key linking subject codes to identifiers will be password-protected kept in on a secure-drive drive behind the school of medicine firewall.

8.0 STUDY MANAGEMENT

8.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical

Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing a IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

8.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided:

- A copy of the official IRB approval letter for the protocol and informed consent
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol College of American Pathologist (CAP) and Clinical Laboratory Improvement Amendments (CLIA) Laboratory certification numbers and institution lab normal values.

8.3 Registration Procedures

All participants will be registered and entered into the web-based clinical research platform, Oncore®.

8.4 Data management and Monitoring/Auditing

Data will be stored in Oncore®, the LCCC Clinical Research Registration Database, which is a secure, password-protected, web-based platform and accessible solely to trained study staff. A secure, password-protected, web-based REDCap database will also be maintained and accessible only to trained study staff.

As an investigator-initiated study, this trial may also be audited by the UNC office of Clinical Research every twelve months.

8.5 Data Safety Plan

All study subjects will receive a coded identifier such as "1930-001" which will be used in all data analysis and records. Only the study coordinator will have the key that associates the patient's identifiable information with the coded identifier, and the key will be kept in a secure location. All image metadata will contain only the coded identifier and will be otherwise anonymized. Lab data will receive only the coded identifier.

Image datasets will be stored on a secure server in the Biomedical Research Imaging Center with patient identifiers removed. De-identified copies of these may be stored temporarily on password-protected computers or portable hard drives for use in data analysis. Quantitative data extracted from the images, results of laboratory analysis, and results of data analysis will be maintained on a secure password-protected computer. All data will be archived on a password-protected computer in the office of the PI and backed up with an automated system. Datasets will be maintained for at least five years from the close of the study.

8.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

8.5.1 Emergency Modifications

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC IRB approval.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

8.5.2 Single Patient/Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

8.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the

investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs please follow the guidelines below:

Protocol Deviations: UNC personnel will record the deviation in OnCore® (or other appropriate database set up for the study), and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

Unanticipated Problems:
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB (see section 6.3.1) must be reported by the Study Coordinator using the IRB's web-based reporting system.

8.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

8.7 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction

of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until five years after the completion and final study report of this investigational study.

8.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

9.0 REFERENCES

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