Determining the Magnitude of Change in [^{18}F]FPIA-detectable Short Chain Fatty Acid Uptake in Metastatic Renal Cell Cancer Following Therapy

Short Title: mRCC therapy response by FPIA PET/CT

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Protocol authorised by:

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Sponsor:

Imperial College London is the main research Sponsor for this study.

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This protocol describes the 'Determining the Magnitude of Change in [^{18}F]FPIA-detectable SCFA Uptake in Metastatic Renal Cell Cancer Following Therapy' and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator. This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Clinical Queries: Clinical queries should be directed to Dr Naveed Sarwar who will direct the query to the appropriate person

Investigator Protocol Agreement Page

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct.
- I agree to personally conduct or supervise the described clinical study in accordance with Good Clinical Practice requirements.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations.
- I authorise this protocol.

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1 LIST OF ABBREVIATIONS and TERMS

Abbreviation	Explanation
AE	Adverse Event
CI	Chief Investigator
CIF	Clinical Imaging Facility
CECT	Contrast Enhanced Computerised Tomography
CT	Computerised Tomography
ccRCC	Clear Cell Renal Cell Cancer
ECOG	Eastern Cooperative Oncology Group
ESMO	European Society for Medical Oncology
[^{18}F]FPIA	[^{18}F]Fluoropivalate
[^{18}F]-FDG	[^{18}F fluorodeoxyglucose
GMP	Good Manufacturing Practice
HRMS	High Resolution Mass Spectrometry
Hb	Haemoglobin
ICH GCP	International Conference on Harmonisation – Good Clinical Practice
IO	Immunotherapy
irRECIST	Immune-related response evaluation criteria in solid tumours
LC	Liquid chromatography
mL	Millilitre
mm	Millimetre
mRCC	Metastatic Renal Cell Cancer
PERCIST	Positron Emission Tomography Response Criteria in Solid Tumours
PET	Positron Emission Tomography
PIC	Participant Identification Centre
RCC	Renal Cell Cancer
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
SAE	Serious adverse event
SCFA	Short-chain Fatty Acid
TKI	Tyrosine Kinase Inhibitor
TMB	Tumour Mutational Burden

2 SYNOPSIS

Title	**Determining the Magnitude of Change in [^{18}F]FPIA-detectable SCFA Uptake in Metastatic Renal Cell Cancer Following Therapy**
Phase type	Explorative biomarker investigation / Phase 2
Objectives	**Primary:** • Determine the magnitude of change in [^{18}F]FPIA-detectable SCFA uptake in mRCC following therapy. **Secondary:** • To determine how [^{18}F]FPIA-detectable SCFA uptake in healthy tissue compares to tumour • To determine if changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC at 4-6 weeks and at 12 weeks (± 4 weeks) are related to routinely measured imaging changes. **Tertiary:** • To investigate if baseline or changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC correlate with mutational signature. • To investigate if baseline or changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC correlate with serum and/or urine carnitine/carnitine ester signature.
Endpoints	**Primary:** • Quantitative longitudinal measurement of [^{18}F]FPIA uptake in mRCC. **Secondary:** • Quantitative measurement of [^{18}F]FPIA in healthy tissue including kidneys • Comparison of [^{18}F]FPIA relative to baseline at 4-6 weeks and at 12 weeks (± 4 weeks) to changes on a patient's routine imaging scan e.g. CT chest-abdo-pelvis (by RECIST 1.1), Whole body FDG-PET (by PERCIST), ultrasound or MRI. **Tertiary:** • [^{18}F]FPIA uptake *versus* Tumour Mutational Burden (TMB) on biopsy material. • [^{18}F]FPIA uptake *versus* serum and urine carnitine/carnitine ester signature.
Sample Size	24 In the event of withdrawals or unevaluable data, additional patients will be recruited to reach a total number of 24 evaluable subjects.

Inclusion Criteria	Patients with radiological and/or histological evidence of evidence of mRCC who are either: A. Treatment naïve or newly relapsed (not currently on treatment) or B. Progressing on standard of care systemic therapy and C. that fulfil the following criteria: 1. Age ≥18 2. Target metastases size ≥ 1cm which is not in the liver. 3. The subject has an available diagnostic tumour biopsy of the primary and/or metastatic lesion taken prior to the first [^{18}F]FPIA PET/CT. 4. WHO performance status 0 – 2. 5. If female, the subject is either post-menopausal (>1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy, >2 years), or if of childbearing potential, must have a negative urine pregnancy test at initial screening and/or within 2 hours prior to injection of imaging agent. 6. eGFR of ≥30 within 3 months of [^{18}F]FPIA injection 7. The subject is able and willing to comply with study procedures, and a signed and dated informed consent is obtained. 8. The subject is not scheduled to start cancer treatment prior to the first study PET/CT scan.
Exclusion Criteria	1. Pregnant or lactating women 2. Evidence of significant medical condition or laboratory finding which, in the opinion of the Investigator, makes it undesirable for the patient to participate in the trial 3. The subject is receiving or has received chemotherapy, immunotherapy, biologic therapy or investigational therapy within 14 days or five half-lives of a drug (whichever is longer) prior to the first dose of [^{18}F]FPIA injection. 4. The subject is scheduled to have a routine nuclear medicine or contrast scan the same day prior to the administration of [^{18}F]FPIA.

	5. Participants with claustrophobia or who are unable to comfortably tolerate the scanning procedure.
Intervention	Subjects will receive a single I.V. bolus injection of [^{18}F]FPIA over a period of about 30 seconds (for each visit). The tracer injection will be followed by a saline flush. Patients will receive a maximum ^{18}F activity of 370 MBq. As part of this study the subject will have three [^{18}F]FPIA PET/CT scans. The first will be before the start of cancer treatment, the second 4-6 weeks after start of cancer treatment, and the third about 12 weeks after start of cancer treatment.
Statistical Methods and Planned Analysis	The study is an Observational Non-randomised Phase 2 study to determine the magnitude of change in [^{18}F]FPIA-detectable SCFA uptake in mRCC using [^{18}F]FPIA PET/CT following therapy. This is the first time we are using [^{18}F]FPIA in patients with mRCC, thus we do not know *a priori* the magnitude of uptake. Statistical tests will use a 0.05 significance level and will be 2-sided unless otherwise noted. 24 evaluable patients will be recruited for the study with the Upper Confidence Interval of sensitivity to detect [^{18}F]FPIA-detectable SCFA uptake set to $\geq$ 90%.

3 BACKGROUND AND RATIONALE

3.1 Background

The morphologically and genetically heterogeneous disease – Renal cell carcinoma (RCC) – is the most prevalent and lethal malignancy of the kidney; non clear cell RCC especially Papillary RCC has a worse prognosis than Clear Cell RCC (ccRCC). At the time of diagnosis, as many as a fifth of patients have metastatic disease (mRCC). RCC has exhibited resistance to chemotherapy and radiation. Despite the evolution of tyrosine kinase inhibitors (TKIs) and immunotherapy (IO), long-term survival rates remain poor. Importantly, there are no predictive biomarkers although the International Metastatic RCC Database Consortium (IMDC) risk score is increasingly being used to stratify patients to their systemic therapy (see European Society for Medical Oncology (ESMO) guidelines[1]). Furthermore, useful imaging biomarkers of response in RCC are lacking, with [^{18}F]FDG-PET which looks at glucose metabolism, and ^{124}I-cG250 -PET which targets carbonic anhydrase IX in VHL mutated RCC subtypes such as ccRCC, being ones that have been extensively explored[2]. Notably, response in bone metastases are notoriously difficult to assess.

We have developed a new tracer, ^{18}F-fluoropivalate ([^{18}F]FPIA) that images short chain fatty acid (SCFA) uptake in tumours, a key component of fatty acid oxidation[3,4]. Like its non-radioactive analogue[5], [^{18}F]FPIA is converted to FPIA-carnitine upon injection likely in the liver[4] and is hypothesised to be transported via the blood to the tumour where it accumulates[3,4]. In collaboration with Invicro, we have conducted GMP manufacturing and first-in-human evaluation[4]. We are currently conducting studies in brain tumours due to the low background of this radiotracer in healthy brain compared to brain tumours, unlike FDG. Here we propose to study the [^{18}F]FPIA-detectable tumour SCFA uptake in RCC and the effect of therapy on the signal. There is paucity of data on SCFA uptake, carnitine metabolism and related transporter activity in RCC, but there are strong suggestions that this may be a highly relevant biology of RCC. By extracting surgically removed normal kidney, ccRCC and urine followed by Liquid chromatography- High Resolution Mass Spectrometry (LC-HRMS), Niziol J and co-workers recently reported that SC-carnitine metabolites including hydroxybutyrylcarnitine and propanoylcarnitine, as well as carnitine were in much higher concentrations in RCC compared to paired normal tissues; and in urine of cancer patients compared to healthy controls[6]. This may be due in part to import of these metabolites from systemic circulation into tumour (and renal elimination) rather than increased synthesis within tumours, since enzymes for synthesis in tumour are downregulated in RCC[7]. Furthermore, effective treatment that alters the metabolic capacity of RCC should decrease import of SCFA, hence [^{18}F]FPIA uptake. The main therapeutic options for RCC patients are TKIs, including Sunitinib, Axitinib, Tivozanib, Cabozantanib and Pazopanib, and more recently immunotherapy, including Pembrolizumab, Avelumab, Ipilimumab and Nivolumab, which can present with pseudo progression/pseudo response phenotypes due to changes in vascular physiology/perfusion or immune cell infiltration. Patients receiving these therapies as part of their routine care, who are being monitored by standard Response evaluation criteria in solid

tumours/ *Immune-related* Response evaluation criteria in solid tumours (RECIST/PERCIST/irRECIST) will be studied.

3.2 Rationale

Hypothesis: Import of [^{18}F]FPIA-detectable SCFA into tumours is high and decreases with effective treatment.

Aim: To investigate longitudinal changes in [^{18}F]FPIA uptake at baseline, and at 4-6 weeks (1-2 cycles) and 12 weeks (± 4 weeks); (routine CECT evaluation timepoints) following treatment initiation in patients using TKIs, chemotherapy, IO, or combinations of these.

4 STUDY OBJECTIVES AND ENDPOINTS

4.1 Primary:

Primary Objective	Primary Endpoint
Determine the magnitude of change in [^{18}F]FPIA-detectable SCFA uptake in mRCC following therapy.	Quantitative longitudinal measurement of [^{18}F]FPIA uptake in mRCC.

4.2 Secondary:

Secondary Objectives	Secondary Endpoint
To determine how [^{18}F]FPIA-detectable SCFA uptake in healthy tissue compares to tumour.	Quantitative measurement of FPIA in healthy tissue including kidneys.
To determine if changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC at 4-6 weeks and at 12 weeks (± 4 weeks) are related to routinely measured imaging changes.	Comparison of [^{18}F]FPIA relative to baseline at 4-6 weeks and at 12 weeks (± 4 weeks) to changes on a patient's routine imaging scan e.g. CT chest-abdo-pelvis, Whole body FDG-PET, ultrasound or MRI (RECIST 1.1 or irRECIST or PERCIST.

4.3 Tertiary:

Tertiary Objective	Tertiary Endpoint
To investigate if baseline or changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC correlate with mutational signature.	[^{18}F]FPIA uptake *versus* Tumour Mutational Burden (TMB) on biopsy material.
To investigate if baseline or changes in [^{18}F]FPIA-detectable SCFA uptake in mRCC correlate with serum or urine carnitine/carnitine ester signature.	[^{18}F]FPIA uptake *versus* serum or urine carnitine/carnitine ester signature.

5 STUDY PLAN AND PROCEDURE

5.1 Study Overview

24 evaluable patients (all IMDC risk groups; metastatic ccRCC or other subtypes) with radiological and/or histological evidence of mRCC and due to receive physician choice of systemic therapy will be enrolled. The patients invited to participate in the study will provide written informed consent, but will only undergo [^{18}F]FPIA PET/CT imaging once they have satisfied the inclusion and exclusion criteria. Once these have been satisfied, eligible patients will proceed to [^{18}F]FPIA PET/CT. Patients will have three imaging visits. Data will be considered complete when patients have all three analysable scans. In the event of dropout, additional subjects will be recruited to reach a total number of 24 evaluable subjects.

On each day of imaging the patients will have a blood test and a urine sample collected to measure concentrations of carnitine. For each scan, a single dose of [^{18}F]FPIA (maximum, 370 MBq) IV will be administered to the participant. The participant will then rest in a quiet place for an uptake period & undergo whole body PET/CT scanning from 60 min (vertex to knees).

Archival tumour biopsies (primary or metastatic lesion), taken prior to the 1st [^{18}F]FPIA PET/CT scan, will be retrieved for analysis where possible.

All patients will have 3 [^{18}F]FPIA PET/CT scan visits on different days:

Scan 1: Baseline scan to be conducted prior to initiating standard of care therapy.

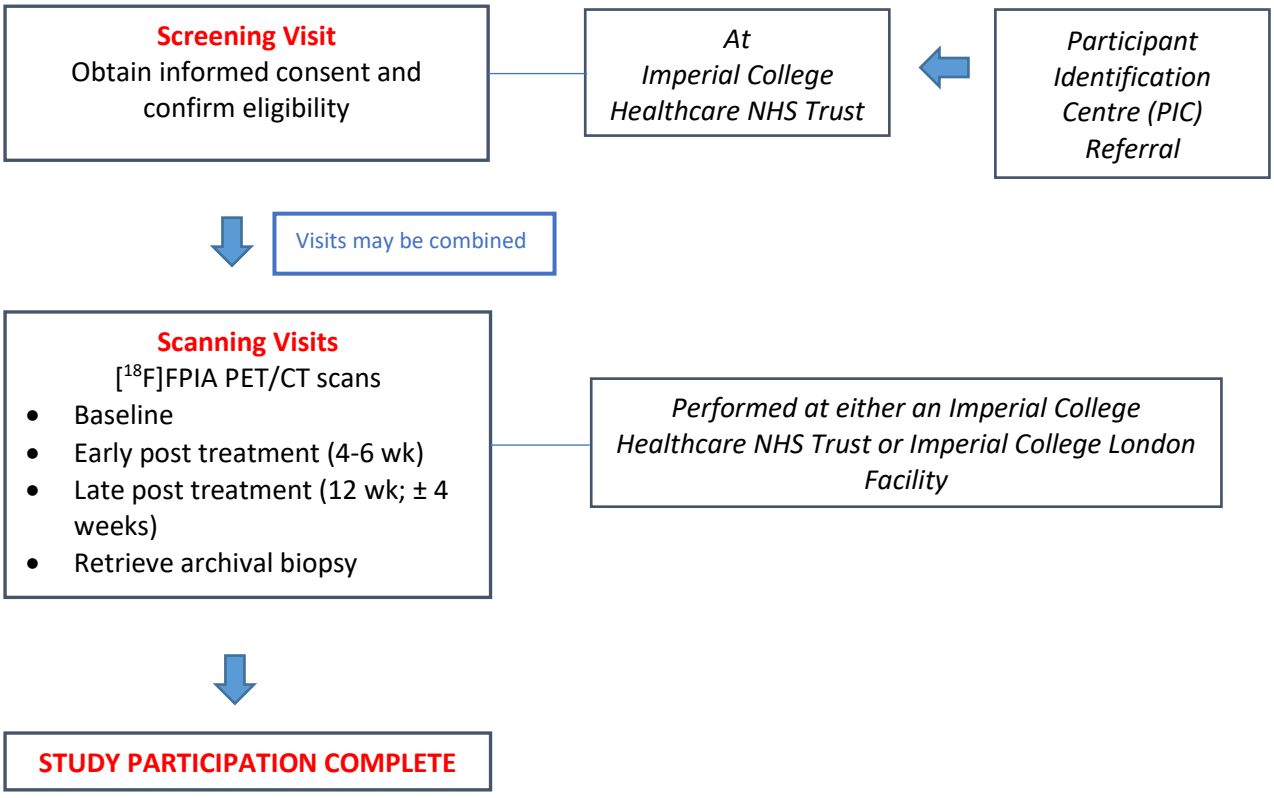
Scan2: Early post-treatment scan to be conducted at 4-6 weeks after initiating therapy.

Scan3: Late post-treatment scan to be conducted at 12 weeks (± 4 weeks) after initiating therapy.

Drug therapy pre study: Patients will have been treated as per standard of care, which may have included TKIs, chemotherapy, IO or combinations of these. 1st line and 2nd line patients can be included in the study after washout period of 14 days or 5 half-lives of drug (whichever is longer) providing they fit all inclusion and exclusion criteria.

Drug therapy on study: Patients will be treated as per standard of care, which may include TKIs, chemotherapy, IO and combinations of these.

5.2 Study Flow Chart



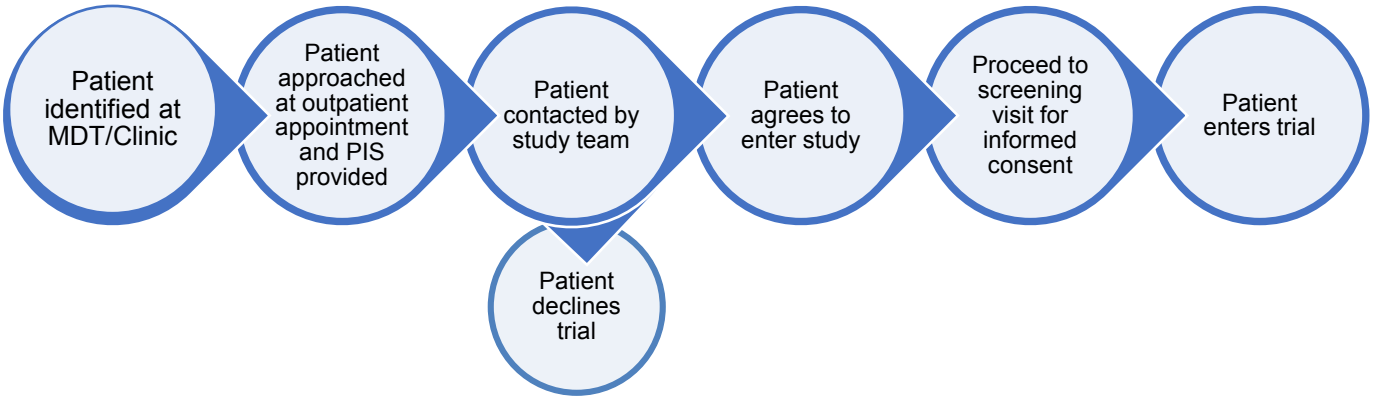
6 PARTICIPANT ENTRY

A target of 24 evaluable subjects will be recruited.
In the event of withdrawals or non-evaluable data before or after dosing, additional subjects will be recruited to reach a total number of 24 evaluable subjects. Evaluable data from patients who drop out will be used whenever possible.

6.1 Identification and recruitment of subjects

Potentially eligible patients will be identified from the Imperial oncology multidisciplinary team meeting and from outpatient clinics by a member of the clinical and research team. The treating clinician will discuss the study with the patient at their clinic appointment and a patient information sheet explaining the study will be given to them or agreed to email or post to them either by the clinician or by a member of the clinical research team. Patients will be given a minimum of 24 hours to consider participation in the study. After this time, they will be contacted by a member of the research team to discuss the study in more detail. Written and dated informed consent will be obtained prior to any protocol-specific procedures being performed. Patients will then be invited to attend a screening visit. The screening visit may be on the same day as the baseline scan.

Figure 2. Recruitment pathway



6.2 Screening

Each patient will undergo screening assessments to confirm eligibility. Evaluations of the tumour and other clinical data obtained as standard of care prior to consent may be used for the study, provided they comply with the protocol specified timelines. Written informed consent will be obtained before the patient undergoes any study specific procedures.

Each potential patient will be assigned a unique identifier number for use during the trial. A complete record of all patients who enter screening for the study, as well as those who go on to be enrolled must be maintained at each site.

Eligible patients who take part in the study must meet all of the listed inclusion criteria and none of the exclusion criteria.

6.3 Inclusion Criteria

Patients who meet all of the following inclusion criteria will be considered eligible for this study:

Patients with radiological and/or histological evidence of evidence of mRCC who are either:

A. Treatment naïve or newly relapsed (not currently on treatment)

or

B. Progressing on standard of care systemic therapy

and

C. That fulfil the following criteria:

1. Age ≥18
2. Target metastases size ≥ 1cm which is not in liver.
3. The subject has an available diagnostic tumour biopsy of the primary and/or metastatic lesion taken prior to the first [^{18}F]FPIA PET/CT.
4. WHO performance status 0 – 2.
5. If female, the subject is either post-menopausal (>1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy, >2 years), or

if of childbearing potential, must have a negative urine pregnancy test at initial screening and/or within 2 hours prior to injection of imaging agent.

6. eGFR of ≥30 within 3 months of [^{18}F]FPIA injection

7. The subject is able and willing to comply with study procedures, and a signed and dated informed consent is obtained.

8. The subject is not scheduled to start cancer treatment prior to the first study PET/CT scan.

6.4 Exclusion Criteria

Patients who meet any of the following exclusion criteria will not be considered eligible for this study:

1. Pregnant or lactating women

2. Evidence of significant medical condition or laboratory finding which, in the opinion of the Investigator, makes it undesirable for the patient to participate in the trial

3. The subject is receiving or has received chemotherapy, immunotherapy, biologic therapy or investigational therapy within 14 days or five half-lives of a drug (whichever is longer) prior to the first dose of [^{18}F]FPIA injection.

4. The subject is scheduled to have a nuclear medicine or contrast scan within 24 hours of the administration of [^{18}F]FPIA.

5. Participants with claustrophobia or who are unable to comfortably tolerate the scanning procedure.

6.5 ID Assignment

Subject numbers will be assigned in successive order of inclusion.

7 STUDY PROCEDURES

7.1 Table 1 – participant procedures

	-- 28 to 0 days	Scan 1 (Baseline pre-treatment)					Scan 2 (4-6 weeks post treatment) Scan 3 (12 weeks post treatment; +/- 4 weeks)				
	Screening	Pre-PET/CT 120mins	Tracer Dose 0 mins	Low Dose CT	Whole-body PET sweep[6] 60 min	Post scan 24 hrs	Pre-PET/CT 120mins	Tracer Dose 0 mins	Low Dose CT	Whole-body PET sweep[6] 60 min	Post scan 24 hrs
Informed Consent	•										
Entry Criteria	•										
Medical History	•										
Treatment History[1]	•	•					•				
Height[2] / Weight		•					•				
Pregnancy Test[3]		•					•				
WHO Performance status	•										
Renal function eGFR[4]	•										
Carnitine (blood)[5]		•					•				
Carnitine (urine)[6]		•					•				
[^{18}F]FPIA injection			•					•			
PET scanning[7]					•					•	
Adverse events[8]		•	•	•	•	•	•	•	•	•	•
Tumour Biopsy[9]		→	→	→	→	→	→	→	→	→	→

1. Cancer treatment history.
2. Repeat Height measurement not required at scans 2 and 3.
3. In women of child bearing potential. On scan day, must be carried out within 2 hours prior to injection of imaging agent.
4. Review of standard of care eGFR bloods taken within 3 months of [^{18}F]FPIA injection or new sample (≤6mls).
5. Approximately 15 mls
6. Approximately 20mls
7. PET/CT scanning protocol is the same for all scans. Includes low dose CT for anatomical localisation and attenuation correction of the PET data.
8. Adverse events related will be collected for each 24 hour period post each PET/CT scan.
9. A diagnostic tissue biopsy of the primary and/ or metastatic lesion obtained prior to the 1st scan will be retrieved for analysis at any time during study participation.

7.2 Screening Period

Subjects will be screened up to 28 days before tracer administration to confirm eligibility.

Signed and dated informed consent must be obtained from all subjects prior to any protocol specific procedures being performed.

7.3 Screening Assessments

7.3.1 Demographic Data and Medical History

Demographic data and other characteristics will be recorded and will include date of birth, height, weight.

A standard medical history will be obtained including details of previous cancer treatment, surgical history and tumour characteristics.

7.3.2 WHO Performance Status

Performance status will be assessed at the scheduled time points indicated in Table 1 according to WHO criteria as follows:

Table 2:

0	Fully active, able to carry on all pre-disease activities without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair 50% or more of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Death

7.3.3 Pregnancy Test

A urine pregnancy test will be performed within 2 hours prior to injection of the imaging agent in women of childbearing potential.

7.3.4 Laboratory Evaluations

Blood samples for renal function may have been taken as standard of care in this patient population. Results of blood tests taken within 3 months of the scan can be used for study purposes. A new blood sample will be taken if a result is not available (aproximately 6 mls).

New blood and urine samples will be taken for carnitine testing prior to each PET/CT scan.

7.3.5 Tumour Biopsy

A diagnostic tissue biopsy of the primary and/ or metastatic lesion obtained prior to the 1st scan will be retrieved for analysis of TMB.

7.3.6 Follow Up period

There is no study specific FU following the final research PET/CT. AEs will be reportable for up to 24 hours post each scan. The patients will be assessed and followed up in all routine clinical procedures as per local policy by the treating clinician.

8 COMPLETION, WITHDRAWAL AND TERMINATION CRITERIA

8.1 Completion

8.1.1 Definition of completed subject

A completed subject is one who has completed all imaging sequences of the combined [^{18}F]FPIA PET/CT protocol.

8.1.2 Definition of an evaluable subject

An evaluable subject is one who has completed study and where the images from the [^{18}F]FPIA PET/CT are of sufficient quality for analysis.

In the event of withdrawals or non-evaluable data before or after dosing, additional subjects will be recruited to reach a total number of 24 evaluable subjects.

8.1.3 Time on study

The last study related visit for a subject would be the final research PET/CT scan. There are no follow up patient visits unless follow up for resolution of Adverse Events is required.

Depending on the screening period taken in relation to the scan day and approximating a treatment start within 4 weeks of the baseline scan, a subject can be on study between 12 and 21 weeks plus a 24 hour post scan reportable Adverse Event period.

8.2 Rules for subject withdrawal

There are no formal withdrawal criteria for this study. During the conduct of the study, the study team will review the safety data for trends and signals that would indicate the need for withdrawal of a subject.

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time. Investigator(s) also have the right to withdraw subjects from the study in the event of illness, AEs, or other reasons concerning the health or well-being of the subject, or in the case of lack of co-operation. Should a subject decide to withdraw after administration of the tracer, or should the investigator decide to withdraw the subject, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A complete final evaluation at the time of the

subject's withdrawal will be made and an explanation given of why the subject is withdrawing or being withdrawn from the study.

Subjects who discontinue standard of care treatment before scan visit 2 will not proceed to scan 2 or scan 3.

Subjects who discontinue standard of care treatment between scan visit 2 and scan visit 3 will be reviewed by the investigator with regard to proceeding to scan visit 3 depending on proximity of treatment cessation to final scan window.

The reason for non-completion and the date and time of the last contact with the subject must be noted in the research documentation. If the reason for withdrawal is a clinical AE or an abnormal laboratory result, monitoring will continue until the outcome is evident. The specific event or test result(s) must be recorded in the research documentation.

8.3 Rules for terminating study

The sponsor reserves the right to terminate the study at any time. Prior to dosing of the first subject, the study may be terminated by the sponsor and the investigator without consultation with the Administration of Radioactive Substances Advisory Committee (ARSAC) holder and Ethics Committee. The ARSAC holder, Ethics Committee and Health Research Authority (HRA), must be promptly notified that the study will no longer be taking place and provided with a detailed written explanation. Once dosing with radiopharmaceutical has begun, the study may only be terminated if a careful review of the overall risk benefit analysis demonstrates that the assumptions have changed and that the overall balance is no longer acceptable. The investigators will temporarily halt the study if there has been a SAE which has been judged to be related to the study dosing. In these circumstances, termination can only take place with the agreement of the ARSAC holder and the Ethics Committee.

If it becomes necessary to consider termination of the study after dosing has begun, dosing may be suspended pending discussion between the sponsor, the investigator, the ARSAC holder and the Ethics Committee. Dosing may always be immediately suspended for safety reasons.

8.4 End of Study

The end of the study is defined as the last visit of the last patient undergoing the study.

9 RADIOTRACER AND IMAGING

9.1 Radiotracer product details

[^{18}F]FPIA is a radiotracer which is used in PET imaging. All PET imaging and therefore all handling of [^{18}F]FPIA will occur at either an Imperial College Healthcare NHS Trust or Imperial College London Facility. Recruitment sites will not handle [^{18}F]FPIA.

9.1.1 Supply Packaging and Labelling

Due to the short half-life of [^{18}F]FPIA, supply will be on a per-patient basis.

[^{18}F]FPIA will be packaged, labelled and distributed by an appropriate courier to sites by the University College London GMP facility.

Labels will be prepared in accordance with Good Manufacturing Practice Annexe 13 requirements and local regulatory guidelines.

9.1.2 Dispensing and Accountability

[^{18}F]FPIA will be dispensed as per local practice at the Imperial College London or Imperial College Healthcare NHS Trust Facility and only by staff authorised to do so by the principal investigator.

[^{18}F] activity will be measured using a calibrator according to the working instructions at the site. Volumes will be adjusted to avoid counter saturation.

Unused [^{18}F]FPIA will be reconciled, accounted for and disposed of by the Imperial College London or Imperial College Healthcare NHS Trust Facilities according to local guidelines.

Radiotracer accountability records will be maintained throughout the course of the study and filed with delivery documentation.

The investigators are responsible for ensuring that deliveries of the tracer and study material are correctly received, recorded, handled and stored safely and properly, in accordance with regulatory guidelines (ICH-GCP and Good Manufacturing Practice (GMP) and used in accordance with this protocol.

9.2 Imaging Protocol

[^{18}F]FPIA PET/CT imaging will occur at an Imperial College Healthcare NHS Trust or Imperial College London Facility

Patients taking part in this study will have whole body [^{18}F]FPIA PET/CT imaging on each of 3 scan visits (baseline/4-6 weeks post therapy/ 12 weeks post therapy (± 4 weeks)). Patients will receive a dose of [^{18}F]FPIA on each of the 3 scan days.

- Patients will attend the site imaging facility on the scan day.
- Pre-scan assessments will be carried out as per Table 1.
- A small cannula will be inserted into a vein in an arm and a blood sample taken for carnitine testing.
- At 60 minutes prior to the planned PET scan start time, the patient will be injected with a maximum administered activity 370 MBq [^{18}F]FPIA in a suitable upper limb vein. The administration will be followed by a saline flush.
- After an uptake period of approximately 50 minutes, the patient will be asked to void urine and made comfortable on the scanner bed.
- At approximately 55minutes after injection of [^{18}F]FPIA, the patient will undergo a low-dose whole body CT scan, (skull vertex to knees) for attenuation correction purposes and to facilitate accurate anatomical localization.
- They will undergo whole-body (skull vertex to knees) PET imaging at 60 minutes. For follow up scans the timing will be as for visit 1 +/- 5 minutes.
- The cannula will be removed at the end of the scan
- The actual dose of [^{18}F]FPIA administered will be recorded in the study notes.
- AEs will be reportable for up to 24hours after the scan.
- The imaging acquisition will be the same for all [^{18}F]FPIA PET/CT imaging sessions.

9.3 Radiation dosimetry

The total ionising radiation exposure will be from both PET and CT scans combined. Participants will have three [^{18}F]FPIA PET/CT scan visits.

Table 3:

Tracer	**[^{18}F]FPIA**	**low-dose CT for attenuation Whole Body (skull vertex to knees)**
Maximum injected dose per scan	370MBq	
Effective dose (mSv/MBq)	0.0154 mSv/MBq	
Effective dose (mSv)/scan	5.7	6.00
Total Research Protocol Dose (TRPD) (3 PET/CT scan visits)	= 35.1 mSv	

Each patient that participates in this study will have 3 PET/CT scans in addition to their standard of care.

The imaging protocol and associated dosimetry is as per Table 3.

Following an uptake period, the participant will undergo a low dose CT for attenuation correction of PET data (with a dose constraint of 6.0 mSv and image acquisition lasting approximately 30 minutes in total). The radiation dose from 370MBq [^{18}F]FPIA will be 5.7 mSv (*Dubash et al. 2020)*. The maximum additional radiation dose from one [^{18}F]FPIA PET/CT will be 11.7 mSv.

The maximum Total Research Protocol Dose (TRPD) from this study is therefore 35.1 mSv.

The radiation dose required by the study is additional to routine clinical care.

For comparison, the average annual natural background radiation dose in the UK is 2.3 mSv. The TRPD incurred in this study can be compared to approximately 15.3 years of naturally occurring background radiation exposure.

The risk of developing cancer as a consequence of taking part in this study is estimated as 0.18% (or 1 in 570). For comparison, the natural lifetime cancer incidence in the general population is about 50% (1 in 2).

The radioactive fluorine in the tracer decays by half every 109.7 minutes which means that little will remain in the body by the time the patient leaves the scanning centre.

9.4 Imaging Complications

There are no immediate complications anticipated from the PET/CT scan except for potential mild bruising at the site of insertion of the peripheral cannula which should resolve within a few days. Rarely, the participant may feel dizzy after the scan due to lying flat, but this is normally short lived

Scans will be stopped at any time during the procedure if the subject is unable to tolerate it.

10 ADVERSE EVENTS

10.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death;
- Is life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;

* "Life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** “Hospitalisation” means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a patient, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

10.2 Reporting of Adverse Events

10.2.1 Non serious AEs

AEs will be collected from the start of each scan visit to up to 24 hours after each PET/CT scan.

Any AEs which remain unresolved at the patient’s last visit in the study should be followed up by the Investigator for as long as medically indicated.

Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance

10.2.2 Serious AEs

An SAE form should be sent to the Chief Investigator with 24 hours. However, relapse, death and/or hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs will be reviewed by the Chief Investigator or a designated medically qualified representative to confirm expectedness and causality.

All SAEs should be reported to the relevant REC where in the opinion of the Chief Investigator, the event was:

- ‘Related’, i.e. resulted from the administration of any of the research procedures; and
- ‘Unexpected’, i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Please send SAE forms to the Sponsor and CI to the below email addresses:

Contact details for reporting SAEs are as follows:

jrco@imperial.ac.uk (Sponsor E-mail)
naveed.sarwar1@nhs.net (CI E-mail)
Tel: 020 331 11403 (CI telephone)

Please send SAE forms to the Sponsor and CI on the above email addresses:

(Mon to Fri 09.00 -17.00)

11 STATISTICS AND DATA ANALYSES

11.1 Statistical Design

The study is an Observational Non-randomised Phase 2 study to determine the magnitude of change in [^{18}F]FPIA-detectable SCFA uptake in mRCC using [^{18}F]FPIA PET/CT following therapy. This is the first time we are using [^{18}F]FPIA in patients with mRCC, thus we do not know *a priori* the magnitude of uptake. Statistical tests will use a 0.05 significance level and will be 2-sided unless otherwise noted. 24 evaluable patients will be recruited for the study with the Upper Confidence Interval of sensitivity to detect [^{18}F]FPIA-detectable SCFA uptake set to $\geq$ 90%.

11.2 Data Analysis

The clinical data will be entered and stored in an appropriate database. An early stage review of tracer uptake after 5 cases will be carried out. A final analysis will be conducted at the end of the study.

The data analysis will consist of completed cases, i.e. all patients who completed the [^{18}F]FPIA scans and 12 weeks (± 4 weeks) of therapy. This is because the primary endpoint of change in uptake of [^{18}F]FPIA from baseline is measured based on the PET/CT scans pre- and post- therapy.

Analysis of the imaging data will be quantified using SUV. The whole body scans will provide response information (change in SUV) on non-target and target lesions combined. Regions of interest (ROIs) corresponding to tumour and healthy tissue will be drawn on PET images and used to derive semi-quantitative uptake parameters. These may include SUVmean (mean of standardized uptake values (SUV) within an ROI) normalised to body weight (bw), SUVmax and SUVpeak; tumour to background ratio.

The analysis will be reviewed by an experienced investigator/ radiologist/ statistician.

The following associations will be determined: between PET and biological measures

- The relationship between PET and cell proliferation determined by histology.
- The relationship between PET and biological drivers of fatty acid metabolism determined by histology.

12 REGULATORY, ETHICAL AND LEGAL ISSUES

12.1 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

12.2 Ethics Approval

The Study Coordination Centre has obtained approval from the West of Scotland Research Ethics Committee (REC) 5 and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The

study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

12.3 Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the HRA/REC for approval as instructed by the Sponsor. Amendments requiring HRA/REC approval may be implemented only after a copy of the all necessary approval letters have been obtained. Imperial College London Sponsor approval is required before any amendments are made with the exception of urgent safety measures. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

12.4 ARSAC Approval

Employer and Practitioner Administration of Radioactive Substances Advisory Committee (ARSAC) certificates will be required for the specified tracer and indication, and both the employer and practitioner must be authorised to use this tracer for research purposes.

The Chief Investigator will ensure that the procedures are compliant with the Ionising Radiation (Medical Exposure) Regulations, and appropriate review by a Medical Physics Expert and Clinical Radiation Expert has been undertaken.

12.5 Informed Consent

The Chief Investigator will:

- Ensure that the right of the participant to refuse to participate without giving reasons is respected.

- Ensure that each patient is given full and adequate oral and written information about the study including the background, purpose and risks/benefits of participation and the risk associated with the radiation exposure from the PET tracer.

- Ensure that each patient is notified that they are free to withdraw from the study at any time without giving reasons and without prejudicing further treatment.

• Ensure that each patient is given the opportunity to ask questions and allowed sufficient time to read and understand the information sheet

• Ensure each patient provides signed, dated informed consent before undergoing any study specific procedure

• Ensure the original copy of the signed, dated Informed Consent Form is stored in the Investigator site file and a copy is also filed in the medical records

• Ensure that each patient receives a copy of the signed, dated Informed Consent Form

12.6 Indemnity

Imperial College London holds negligent harm and non- negligent harm insurance policies, which apply to this study.

12.7 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

12.8 Funding

This study is funded by the Medical Research Council (MRC).

Patient travel expenses to attend study visits will be reimbursed where necessary. Patients will not be paid for taking part in the study.

12.9 Subject Confidentiality

Imperial College London and Imperial College NHS Trust data protection policies will be followed. Subjects personal data will be available only to clinical and research members directly involved in the study conduct. The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Participants will be allocated a unique study identifier code which will be known to the study research team and staff. The study identifier code will be used to pseudonymise samples, results, datasets and scans which would be held electronically on Imperial College London computers .

Subjects' identification data will be required for the recruitment, screening and registration processes. On study documents submitted to the Sponsor, patients will be identified by a trial ID number only. Documents that are not submitted to the Sponsor (e.g. signed informed consent form) will be kept in a strictly confidential file by the investigator.

The Chief Investigator shall permit direct access to patients' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and REC.

The imaging facility staff at Imperial College Healthcare NHS Trust and Imperial College London will have access to limited participants' personal data for registration, IRMER, safety and governance purposes but may need to view the participant's medical records in the event of any study adverse events or emergencies on the scan day.

The subject's GP will be informed of their participation in the study.

The taxi company will be given the name address & contact number of the subject by email, e booking or telephone.

12.10 Material Storage and Analysis.

Blood samples for renal function will be analysed by Imperial College Healthcare NHS Pathology department and identifiable results reported on a secure web-based results reporting system under the Imperial NHS Trust data protection policy, only accessible by NHS contracted staff.

Blood and urine samples for carnitine will be pseudonymised, processed as required and stored at Imperial College London. They will be analysed at Imperial College London and at an external laboratory and will not be identifiable to laboratory personnel.

Urine samples for pregnancy testing will be pseudonymised and tested immediately by research staff.

All above samples will be disposed of after testing according to local policies.

The tumour specimens will be accessed from the Tissue Bank and stored as per Human Tissue Authority (HTA) regulations. All samples will be pseudonymised with the link only being known to the research team. Approval will be sought for tumour tissue sample use according to local guidelines. Tissue will be analysed within Imperial College London. Diagnostic archived tumour samples will be returned to the local tissue bank.

13 DATA MANAGEMENT

13.1 Data Recording and Storage

All data except for the PET/CT scans performed at the Imperial College London Imaging Facility will originate in the NHS. The principal means of data collection from patient visits will be imaging PET/CT data obtained at either Imperial College Healthcare NHS Trust or Imperial College London. Imaging PET/CT data from the Imperial College London Imaging Facility will be uploaded to Imperial CIF XNAT. Other screening visit and follow up data will be collected on paper CRFs. Data will be pseudonymised and then stored on Imperial College secure servers and on encrypted drives in accordance with the data protection act. All data will be linked pseudonymised prior to being analysed and stored for 10 years.

Patient clinical, follow-up and imaging data will then be inputted into an electronic database (excel spreadsheet) held on a secure, password protected computer within Imperial College Healthcare Trust.

All paper/ manual study documentation including those with personal identifiers will be stored in an access controlled locked office within Imperial College London, and the Imaging Facilities at Imperial College Healthcare NHS Trust and Imperial College London

Subject's original consent forms will be kept in the study master file in a controlled entry secure office at Imperial College London (Hammersmith campus). A copy of the consent forms will be kept in a study master file locked in a secure office at CIF for governance purposes and will also be copied to the medical records.

The Investigator must retain essential documents until notified by the Sponsor and at least for ten years after study completion. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. The data for this study will be maintained on a secure, password protected Imperial College Healthcare Trust computer, with only authorised personnel having access to them.

No study document will be destroyed without prior written agreement between the Sponsor and the CI. Should the CI wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

Data and images obtained from scans may be used in an anonymous form for future research, including that carried out by commercial healthcare companies.

13.2 Audits and inspections

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

13.3 Disclosure of Data

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only.

All data information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) is completed.

14 STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the Imaging research office, Imperial College London, GN1, Commonwealth Building, Hammersmith Campus, Du Cane Rd, London, W12 0NN.

15 PUBLICATION AND DISSEMINATION

The results of the study will be written and will be submitted for publication to a suitable peer reviewed journal. Results may be presented at conferences and internally within the Sponsor institution. A final summary of results will be provided to the REC. All data will be anonymised for publication and no identifiable personal data will be used.
Patients will have the opportunity of being informed when data is published, if they wish.

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