Determining the magnitude of early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI

Short title: Measuring fatty acid oxidation in cerebral metastases using [^{18}F]FPIA

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

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This protocol describes the 'Determining the magnitude of early steps of fatty acid oxidation in cerebral metastases using ^{18}F-FPIA PET/MRI' study 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 Frame Work 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 Matthew Williams 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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Investigator Signature		Date

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1 LIST OF ABBREVIATIONS and TERMS

AE	Adverse event
ARSAC	Administration of Radioactive Substances Advisory Committee
CT	Computed Tomography
ED	Effective Dose (i.e., the sum of risk weighted organ absorbed radiation dose used as a measure of stochastic radiation risk)
EC	Ethics committee
FDG	^{18}F – fluorodeoxyglucose
^{18}F	Fluorine with an Atomic Mass of 18
[^{18}F]FPIA	^{18}F-fluoropivalate (^{18}F-fluoro-2,2-dimethylpropionic acid)
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
i.v	Intravenous
MBq	Megabecquerel
PET	Positron emission tomography
SAE	Serious adverse event
SRS	Stereotactic radiosurgery
SUV	Standardized uptake value
MRI	Magnetic resonance imaging
eGFR	Estimated glomerular filtration rate

2 SYNOPSIS

Name of Sponsor: Imperial College London
Name of biomarker: [^{18}F]Fluoropivalate positron emission tomography magnetic resonance imaging (^{18}F-FPIA PET/MRI)
Title of Study: Determining the magnitude of early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI
Study Centres: • Invicro, Hammersmith Hospital Campus, UK • Imperial College London • Imperial College Healthcare NHS Trust, London
Phase of Development: Explorative biomarker use/ Phase 2
Primary Objective: • Determine the magnitude of the early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI. **Secondary objective:** • Investigate the magnitude of tracer-derived biochemical uptake between treated metastases and treatment naïve metastases. **Tertiary objective:** • Comparison of tracer-derived biochemical uptake measure with conventional MRI variables including perfusion imaging and contrast enhancement.
Primary Endpoint: • Quantitative measurement of [^{18}F]FPIA uptake in cerebral metastases. **Secondary endpoint:** • Distribution of [^{18}F]FPIA uptake in lesions which have undergone treatment, with those that are treatment naïve. **Tertiary endpoint:** • Correlation of [^{18}F]FPIA variables with volumetric and functional MRI variables including perfusion.
Selection of Subjects: Inclusion Criteria: Patients with radiological evidence of cerebral metastases on MRI that are either:

A) Treatment naïve*
or
B) SRS *+/- combination* treated**
and
C) that fulfill the following criteria:

1. Age ≥18
2. Target metastases size ≥ 1cm.
3. WHO performance status 0 – 2.
4. If female, the subject is either post-menopausal (at least 1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy for at least 2 years), or if of childbearing potential, must have a negative urine beta human chorionic gonadotropin (β-HCG) pregnancy test done prior to tracer administration.
5. The subject is able and willing to comply with study procedures, and a signed and dated informed consent is obtained.
6. The subject has a satisfactory medical history as judged by the investigator with no significant co-morbidities.
7. The subject's clinical and laboratory tests are within normal limits and/or considered clinically insignificant.

** Treatment Naïve = = patients who have not and will not be undergoing treatment for cerebral metastases at the time of the PET/MRI scan.*

*** SRS+/- combination therapy = patients who have completed the stereotactic radiosurgery (SRS) part of a combination regime enabling a 4-8 week post SRS treatment PET/MRI scan. Combination therapy can be +/- chemotherapy/immunotherapy/targeted therapies.*

Exclusion Criteria:

1. The subject is pregnant or lactating.
2. Any other chronic illness that will or musculoskeletal condition that would not allow comfortable performance of a PET study.
3. Prior use within 14 days of enrolment or concurrent therapy with any other investigational agent.
4. Unsatisfactory renal function (eGFR<30).

5. The subject has non-MRI compatible devices (e.g. a pacemaker, an implantable cardioverter-defibrillator (ICD), a nerve stimulator, a cochlear implant or a drug pump) or implanted material (e.g. non-MRI compatible sternal wires, biostimulators, metals or alloys).
Number of Patients Planned: 24 • In the event of withdrawals or unevaluable data, additional patients will be recruited to reach a total number of 24 evaluable subjects.
Treatment of Subjects: Experimental imaging biomarker tracer: • Subjects will receive a single I.V. bolus injection of [^{18}F]FPIA over a period of about 30 seconds. The tracer injection will be followed by a saline flush. Patients will receive a maximum ^{18}F activity of 370 MBq per scan, and a maximum of 2 scans.
Statistical Methods and Planned Analysis: • The study is an Observational Non-randomised Phase 2 study to determine the magnitude of the early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI. This is the first time we are using FPIA in patients with cerebral metastases, 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 fatty acid metabolism set to $\geq$ 90%.

3 BACKGROUND AND RATIONALE

3.1 Background

Cerebral metastases represent a significant problem for oncological management. It is estimated that 20% to 40% of patients with cancer will develop metastatic cancer to the brain during the course of their illness [1]. The burden of brain metastases impacts on quality and length of survival. Although cerebral metastases may spread from any primary site, most common primary site is the lung, followed by the breast then gastrointestinal sites. Very recent work indicates a striking common metabolic phenotype of brain metastases and glioblastoma to simultaneously oxidise acetate and glucose [3]. This adaptation may be important for meeting the high biosynthetic and bioenergetic demands of malignant growth. Furthermore, it has been shown that the key gene responsible

for this phenotype, ACSS2, permits cells to exist under bioenergetic stress, which is a potential adaptation to therapy resistance [4]. Tumour cells, including brain tumour cells efficiently oxidize fatty acids synthesized from acetate to generate a substantial part of the cell's ATP requirement, as well as NADPH to buffer reactive oxygen species.

There is currently no method for quantifying tissue fatty acid oxidation in humans. Our group has characterized ^{18}F-fluoropivalate ([^{18}F]FPIA; 3-^{18}F-fluoro-2,2-dimethylpropionic acid) as a tracer to report the early steps of fatty acid oxidation in non-clinical studies using PET imaging [4] [5]. ^{18}F-FPIA shows high contrast for imaging brain tumour xenografts implanted subcutaneously in mice [5], and orthotopically in the brain (unpublished) with the magnitude of tumour uptake equivalent to that of conventional tracers such as [^{18}F]FDG but with significantly lower normal brain uptake giving a high signal to noise ratio enabling its use as suitable surrogate biomarker for fatty acid oxidation. Our current objective is to quantify the magnitude of early step fatty acid oxidation in cerebral metastases thought to possess enhanced fatty acid oxidation [3].

Combined hybrid PET/MRI technology permits simultaneous acquisition of PET imaging with conventional MRI sequences including T1, T2/FLAIR (with and without contrast) and perfusion (DSC-MRI). Patients with brain metastases will either be treatment naïve or have undergone radiotherapy and so we will have the opportunity to investigate the degree of fatty acid oxidation between untreated and treated patient groups, as well as differences in fatty acid metabolism between cerebral metastases from different primary tumours. Depending on the outcome of this study, [^{18}F]FPIA PET/MRI may be further investigated, in a longitudinal setting, for assessing response to therapies including intracranial stereotactic radiosurgery in patients with brain metastases.

3.2 Rationale

3.2.1 Aim

The aim of this study is to quantify the degree of early step fatty acid oxidation in cerebral metastases as imaged by [^{18}F]FPIA PET/MRI.

3.2.2 Hypothesis

We hypothesise that FPIA uptake will be higher in metastases that are treatment naïve compared to those that have undergone treatment, in keeping with viable tumour cells having a high propensity to generate ATP and NADPH via fatty acid oxidation under bioenergetic stress.

4 STUDY OBJECTIVES AND ENDPOINTS

4.1 Study Objectives

4.1.1 Primary Objective:

- Determine the magnitude of the early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI.

4.1.2 Secondary Objectives:

- Investigate of the magnitude of tracer-derived biochemical uptake between treated metastases and treatment naïve metastases.

4.1.3 Tertiary Objective:

- Comparison of tracer-derived biochemical uptake measure with conventional MRI variables including perfusion imaging and contrast enhancement.

4.2 Study Endpoints

4.2.1 Primary Endpoint:

- Quantitative measurement of [^{18}F]FPIA uptake in cerebral metastases.

4.2.2 Secondary Endpoints:

- Distribution of [^{18}F]FPIA uptake in lesions which have undergone treatment, with those that are treatment naïve.

4.2.3 Tertiary Endpoint:

- Correlation of [^{18}F]FPIA variables with volumetric and functional MRI variables including perfusion.

5 STUDY OUTLINE

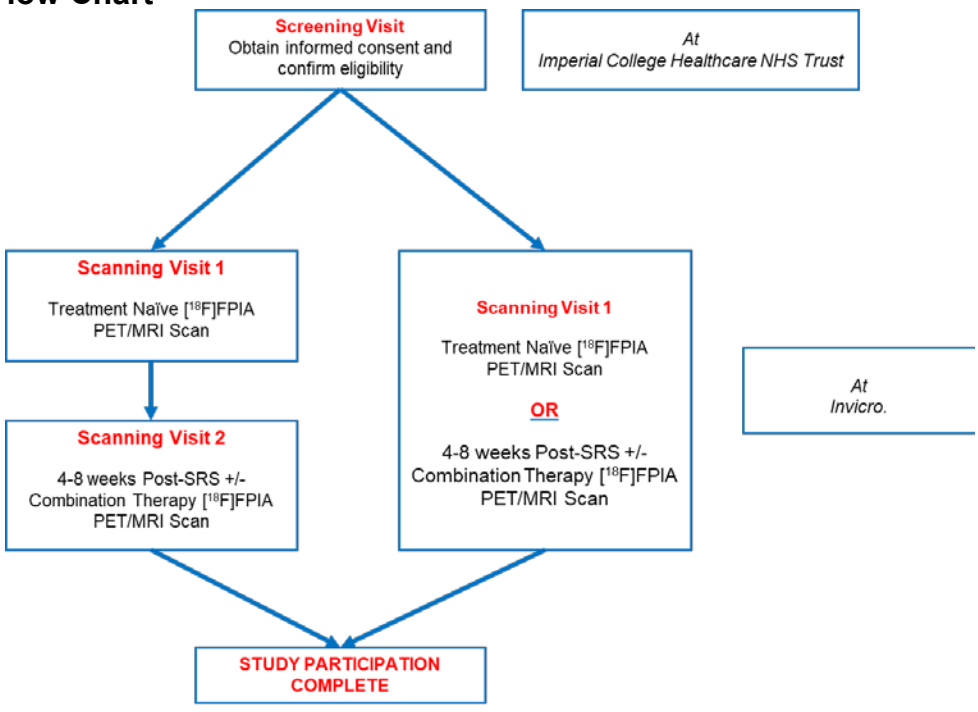
5.1 Study Overview

24 evaluable patients with radiological evidence of cerebral metastases on MRI will be enrolled into the study (12 who are treatment naïve + 12 who have completed SRS+/- combination therapy). The patients invited to participate in the study will provide written informed consent, but will only undergo [^{18}F]FPIA PET/MRI imaging once they have satisfied the inclusion and exclusion criteria.. Once these have been satisfied, eligible patients will proceed to [^{18}F]FPIA PET/MRI. The same treatment naiive patients, who have had one [^{18}F]FPIA PET/MRI scan and completed SRS+/- combination therapy, may be invited to take part in a second [^{18}F]FPIA PET/MRI scan, as long as they satisfy the inclusion and exlusion criteria.

On the day of imaging the patients will undergo a blood test to measure plasma concentrations of carnitine (approximately 6mls). During the scan, a single dose of [^{18}F]FPIA (maximum, 370 MBq) IV will be administered to the participant followed by a whole brain dynamic PET/MRI scan over 66 minutes. During the MRI sequences, the patient will receive a 2 stage IV bolus of Gadolinium contrast medium administered through a peripheral venous cannula.

5.2 Study Flow Chart

Figure 1.



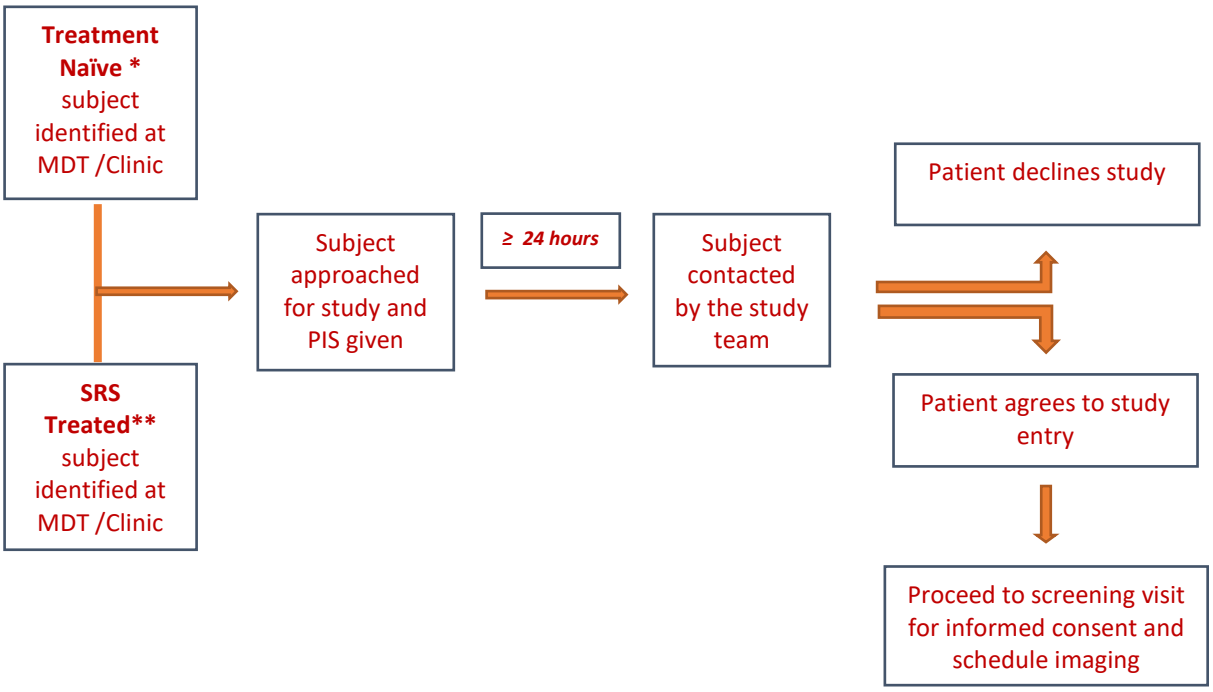
6 PARTICIPANT ENTRY

A target of 24 evaluable subjects will be recruited. In the case of subject withdrawal or non-evaluable subjects before or after dosing, additional subjects will be enrolled *to reach 24 evaluable subjects.*

6.1 Identification and recruitment of subjects

Potentially eligible patients will be identified from the Imperial College Healthcare NHS Trust oncology multidisciplinary team meeting and from outpatient clinics by a members 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 hrs 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. Patients will then be invited to attend a screening up to 21 days before tracer administration and imaging.

Figure 2. Recruitment pathway



*Treatment naïve or **SRS *+/- combination* therapy

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.

6.3 Inclusion Criteria

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

Patients with radiological evidence of cerebral metastases on MRI that are either:

A) Treatment naïve*
   or
B) SRS+/- combination treated**
   and
C) That fulfil the following criteria:

1. Age ≥18.
2. Target metastases size $\geq$ 1cm.
3. WHO performance status 0 – 2.
4. If female, the subject is either post-menopausal (at least 1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy for at least 2 years), or if of childbearing potential, must have a negative urine beta human chorionic gonadotropin (β-HCG) pregnancy test done prior to tracer administration.
5. The subject is able and willing to comply with study procedures, and signed and dated informed consent is obtained.
6. The subject has a satisfactory medical history as judged by the investigator with no significant co-morbidities.

7. The subject's clinical and laboratory tests are within normal limits and/or considered clinically insignificant.

** Treatment Naïve = patients who have not and will not be undergoing treatment for cerebral metastases at the time of the PET/MRI scan*

*** SRS +/-combination therapy = patients who have completed the stereotactic radiosurgery (SRS) part of a combination regime enabling a 4-8 week post SRS treatment PET/MRI scan. Combination therapy can be +/- chemotherapy/immunotherapy/targeted therapy.*

6.4 Exclusion Criteria

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

1. The subject is pregnant or lactating.
2. Any other chronic illness that will or musculoskeletal condition that would not allow comfortable performance of a PET study.
3. Prior use within 14 days of enrolment or concurrent therapy with any other investigational agent.
4. Unsatisfactory renal function (eGFR<30).
5. The subject has non-MRI compatible devices (e.g.a pacemaker, an implantable cardioverter-defibrillator (ICD), a nerve stimulator, a cochlear implant or a drug pump) or implanted material (e.g. non-MRI compatible sternal wires, biostimulators, metals or alloys).

*** A previous [^{18}F]FPIA PET/MRI scan as part of the treatment naiive arm, is not an exlclusion for a second [^{18}F]FPIA PET/MRI scan in the post SRS arm.*

6.5 ID Assignment

Subject numbers will be assigned in successive order of inclusion.

7 STUDY PROCEDURES

The patient procedures are summarized in Table 1 below.

7.1 Schedule of Events

Table 1.

	Screening - 21 to day 0	Pre-PET/MRI day 0	[^{18}F] FPIA Dose	PET/MRI	
Informed Consent	•				
Entry Criteria	•				
Demographic Information	•				
Medical History	•				
WHO performance status	•				
Height and Weight		•			
Prior/Concomitant Medication [1]	•	•			
Blood test for renal function[2]	•				
Carnitine blood test		•			
Pregnancy Test[3]		•			
Vital Signs		•			
[^{18}F]FPIA injection			•		
Gadolinium contrast injection /kg (in **2 stages)**				•	
Simultaneous PET/MRI Imaging*			→		
Adverse events[4]			•	•	•

- [1] If screening and PET/MRI are on same day – procedures do not have to be repeated.
- [2] If result available from routine test within 90 days of [^{18}F]FPIA injection, this does not need to be repeated (determined by investigator).
- [3] In women of child bearing potential prior to injection of imaging agent.
- [4] 24-hour post scan Adverse Event reporting period.

7.2 Screening Period

Subjects will be screened up to 21 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 medication, 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 prior to injection of imaging agent in women of child bearing 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 90 days of the scan can be used for study purposes. A new blood sample will be taken if a result is not available.
Blood will be taken for carnitine testing prior to the PET/MRI scan.

7.3.5 Concomitant Medications

Any concurrent, or procedural medications or therapy given to or taken by the subject from 2 weeks before inclusion into the study up to the end of the observation period will be recorded in the research notes.

7.3.6 Vital Signs

Blood pressure, pulse and temperature will be recorded.

7.3.7 Follow Up period

There is no PET/MRI related routine follow up. AEs will be reportable for up to 24 hours post 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/MRI 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/MRI are of sufficient quality for analysis.

In the case of subject withdrawal or non-evaluable subjects before or after dosing, additional subjects will be enrolled to reach 24 evaluable subjects.

8.1.3 Time on study

The last study related visit for a subject would be the PET/MRI 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, a subject can be on study between 2 and 23 days including 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.

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. In the case of subject withdrawal before or after dosing (i.e., non-evaluable subjects) additional subjects may be enrolled to reach a total of 24 evaluable subjects.

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 Sponsor, 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 Radiopharmaceutical

The [^{18}F]FPIA radiotracer will be made to GMP standards at the Good Manufacturing Practice (GMP) Facility, University College London.

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

[^{18}F]FPIA will be packaged labelled and distributed by an appropriate courier to Invicro by the GMP Facility, University College London. Labels will be prepared in accordance with Good Manufacturing Practice Annex 13 requirements and local regulatory guidelines.

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

- Patients taking part in this study will have either one or two [^{18}F]FPIA PET/MRI scans
- ^{18}F-FPIA PET/MRI imaging will be carried out at the Hammersmith Hospital site,within Invicro.
- Pre-scan assessments will be carried out as per Table 1.
- A peripheral venous cannula will be inserted within the arm for radiotracer and MRI contrast injections.
- Simultaneous [^{18}F]FPIA PET and MRI whole brain imaging (single bed position over the whole brain from vertex).
- Following completion of imaging, the venous cannula will be removed at the end of the scan and patient will be discharged.
- AEs will be reportable for up to 24 hours after the scan.

9.3 PET Protocol

- For the PET scan, a maximum of 370 MBq [^{18}F]FPIA will be injected followed by a saline flush per scan.
- PET data will be acquired immediately after tracer injection as a single-bed position (single bed position over the whole brain from vertex) with a dynamic imaging scan duration of 66 minutes.

9.4 MRI Protocol

The MRI protocol will include the following sequences:

- Sequences for attenuation correction of PET data (DIXON, zero-TE)
- Volumetric T1-weighted images, pre- and post-contrast administration
- Volumetric T2
- Dynamic susceptibility contrast perfusion (DSC)
- Dynamic contrast enhanced perfusion (DCE)

The exact image acquisition parameters for sequences (e.g. TE, TR, flip angle) are hardware dependent and vary between scanner manufacturers and models, and are therefore not specified here. DWI sequences will be acquired prior to contrast agent administration.

9.5 Radiation dosimetry

The total ionising radiation exposure will be from the PET component of one PET/MRI scan. There is no ionising radiation for the MRI component of the scan.

Table 3: Radiation dose estimation

Tracer	[^{18}F]FPIA PET
Maximum [^{18}F]FPIA injected activity	370 MBq
Conversion factor (mSv/MBq)	0.0154*
Total effective dose (mSv)/ patient for one [^{18}F]FPIA PET scan only	5.7 mSv
Total effective dose (mSv)/patient for two [^{18}F]FPIA PET scans	11.4 mSv

*estimated Effective Dose of 18F-FPIA in humans to be 0.0154 mSv/MBq (Dubash et al, Manuscript in preparation).

9.6 Radiation dose

Each patient that participates in this study will have up to two additional PET/MRI scan in addition to their standard of care. If the patient is having radiotherapy (SRS) as part of their standard of care, the radiation dose estimated is 12-35Gy in 1-5 fractions.

For each PET MRI, simultaneous ^{18}F-FPIA PET and MRI whole brain imaging (vertex to C1 vertebral body) will be performed.

There is no ionizing radiation from the MR component of the study only from the one PET scan.

The maximum additional radiation dose associated with having 1 [^{18}F]FPIA PET scan and is estimated to be 5.7mSv (from an administered activity of 370MBq of [^{18}F]FPIA -which is similar to other common ^{18}F PET imaging tracers).

The radiation dose required by the study is additional to routine clinical care. The total protocol dose is 5.7mSv per scan. This is equivalent to 2 years and 6 months of average natural background radiation in the UK. If you have 2 scans as part of this study, the total dose you will receive is 11.4 mSv, which is equivalant to 5 years of the average natural background radiation in the UK. Ionising radiation can cause cancer which manifests itself after many years or decades. The risk of developing cancer as a consequence of taking part in this study is estimated as 0.03% (1 in 3,510) for one scan, and 0.06% (1 in 1,755) for 2 scans.

For comparison, the natural lifetime cancer incidence in the general population is about 50% (1 in 2).

For the patients having radiotherapy, the radiation dose from the [^{18}F]FPIA PET/MRI scan will be very small compared to radiotherapy.

9.7 Imaging Complications

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

MRI scanners use strong magnets, and therefore the subject will be given a safety questionnaire to complete and the study personnel will ask questions to make sure that you can safely have an MRI scan. As the MRI is noisy the subject will be given ear protection to wear. There is a risk of claustrophobia (fear of being in small places) associated with the scanner.

A contrast agent (Gadolinium) will be used for the MRI scans which is injected intravenously. Some studies have shown gadolinium deposits in the brain and other tissues when using gadolinium contrast agents. There is no evidence that

this has caused any harm to patients and recommendations by the European Medicines Agency to use contrast agents with a lower risk of gadolinium deposition will be followed.

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)
matt.williams3@nhs.net(CI E-mail)
Tel: 020 3311 0733

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 the early steps of fatty acid oxidation in cerebral metastases using [^{18}F]FPIA PET/MRI. This is the first time we are using FPIA in patients (with cerebral metastases), 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 fatty acid metabolism set to $\geq$ 90%.

11.2 PET Analysis

Dynamic PET attenuation corrected images will be reconstructed. Regions of interest (ROIs) corresponding to tumour and normal brain tissue will be drawn on summed images (e.g. 15 – 65 min), and used to generate. The ROIs will be used to derive semi-quantitative uptake parameters. These may include SUV_{mean} (mean of standardized uptake values (SUV) within an ROI) normalised to body weight (bw), SUV_{max} and SUV_{peak}.

11.3 Kinetic Analysis

The kinetics of [^{18}F]FPIA will be explored by visual inspection of regional time activity curves (TACs). If an adequate estimate of radioactivity in arterial plasma can be derived directly from delineation of an ROI in the field of view, then the use of image-derived input functions will be explored, along with modelling techniques such as compartmental analysis and / or graphical approaches to derive quantitative measures of tracer uptake. These may include the net irreversible plasma to tumour transfer constant (Ki, ml $cm^{-3} \cdot min^{-1}$, as well as compartmental analysis-derived [^{18}F]FPIA delivery (K1, $ml \cdot cm^{-3} \cdot min^{-1}$ For comparison, tracer retention will also be estimated from SUV analysis (ratio of SUV at 60 min relative to 5 min).

11.4 MRI Analysis

MRI structural imaging sequences will be used for motion correction of PET data and to define tumour volumes using semi-automated intensity based segmentation algorithms such as those available in the software 'Jim'. Perfusion data will be analysed using custom in-house image analysis tools and other software tools such as MATLAB, FSL, Nordic Neurolab and Tarquin. MRI and PET imaging parameters will be combined to develop novel biomarkers which can be used for tumour characterisation and assessment of treatment response.

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

Study approval has obtained from the South Central – Berkshire Research Ethics Committee (REC) 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 Practioner Administration of Radioactive Substances Advisory Committee (ARSAC) certificates will be required for the specified tracer and indication, and both the employer and practioner 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 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. Patient travel expenses to attend study visits will be reimbursed where necessary or a taxi will be provided.

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.

Invicro. staff 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. This would be for the period of an event only and under the jurisdiction of the study investigator.

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 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.

Carnitine plasma samples will be pseudonymised & stored frozen at Imperial College London. They will be sent to an external hospital laboratory for analysis and will not be identifiable to laboratory personnel.

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

All samples will be disposed of after analysis according to local policies and will not be stored for future use.

If the subject withdraws from the study, samples already taken as part of the research will be destroyed if the subject wishes, but data collected up to the withdrawal will be used.

13 DATA MANAGEMENT

13.1 Data Recording and Storage

All data except for the PET/MRI scan will originate in the NHS. The principal means of data collection from patient visits will be imaging data obtained at Imperial College. This is uploaded fully pseudonymised to a dedicated electronic sharing system . Other screening, visit and follow up data will be collected on paper CRFs and collated on to an electronic database (excel spreadsheet) held on a secure, password protected computer within Imperial College Healthcare Trust, as required. 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.

All paper/ manual study documentation including those with personal identifiers will be stored in an access controlled locked office within Imperial College London and Invicro.

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

Personal identifiable data collected at Invicro for registration, IRMER safety & governance purposes will be stored on an electronic Phase 1 system which is compliant with data protection registration.

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.

16 REFERENCES

[1] Tsao, M. N., Rades, D., Wirth, A., Lo, S. S., Danielson, B. L., Gaspar, L. E., … Chang, E. L. (2012). Radiotherapeutic and surgical management for newly diagnosed brain metastasis(es): An American Society for Radiation Oncology evidence-based guideline. *Practical Radiation Oncology*, *2*(3), 210–225. https://doi.org/10.1016/j.prro.2011.12.004

[3] T. Mashimo *et al.*, “Acetate Is a Bioenergetic Substrate for Human Glioblastoma and Brain Metastases,” *Cell*, vol. 159, no. 7, pp. 1603–1614, Dec. 2014.

[4] Z. T. Schug *et al.*, “Acetyl-CoA Synthetase 2 Promotes Acetate Utilization and Maintains Cancer Cell Growth under Metabolic Stress,” *Cancer Cell*, vol. 27, no. 1, pp. 57–71, Jan. 2015.

[5] T. H. Witney *et al.*, “Preclinical Evaluation of 3-18F-Fluoro-2,2-Dimethylpropionic Acid as an Imaging Agent for Tumor Detection,” *J. Nucl. Med.* , vol. 55, no. 9, pp. 1506–1512, Sep. 2014.