Metabolic flux profiling of brain tumours by the new MR-hyperpolarisation technology

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Protocol short title:	LIFE-GlioB	REC Number: 21/SC/0160
IRAS Number:	294968	
Protocol Version:	4.0, dated 17 Dec 2024	

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Study Sponsors: Jointly sponsored by Cambridge University Hospitals National Health Service (NHS) Foundation Trust and the University of Cambridge

1 Protocol Signatures

I have read and give my approval for the attached protocol entitled 'Metabolic flux profiling of brain tumours by the new MR-hyperpolarisation technology' and agree to abide by all provisions set forth therein.

I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

Chief Investigator

Name: Prof Ferdia Gallagher

Signature: _____

Date: _____

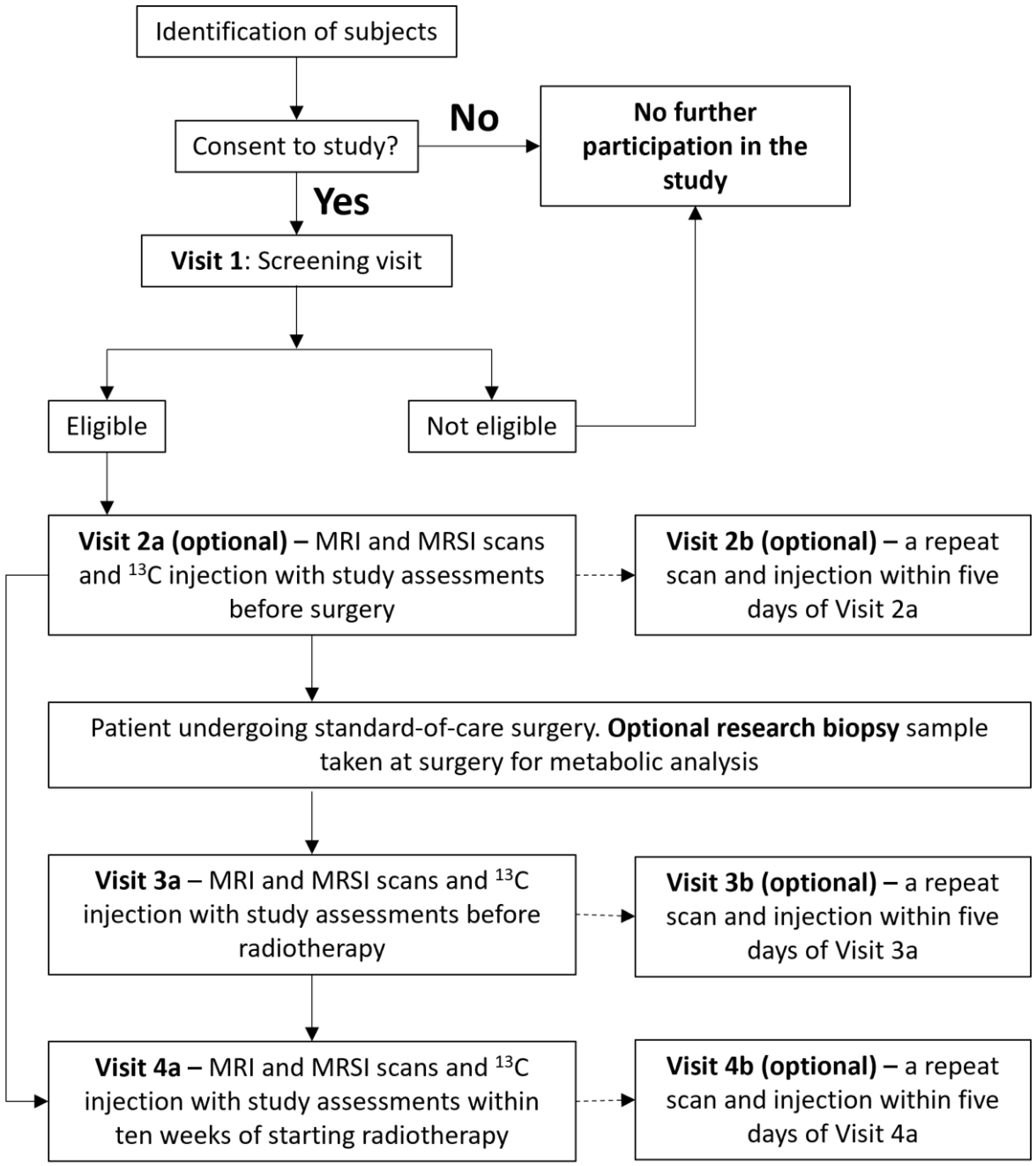
2 Document History

Version	History	Date
1.0	Finalised document created and for sending to ethical review	04 March 2021
1.1	Updated following comments from REC review	27 May 2021
2.0	Updated document created and for sending to ethical review	28 September 2022
3.0	Subject numbers increased for DMI sub study from 11 to 22 Optional CEST sequence added to MRI protocol in additional DMI patients	20 May 2024

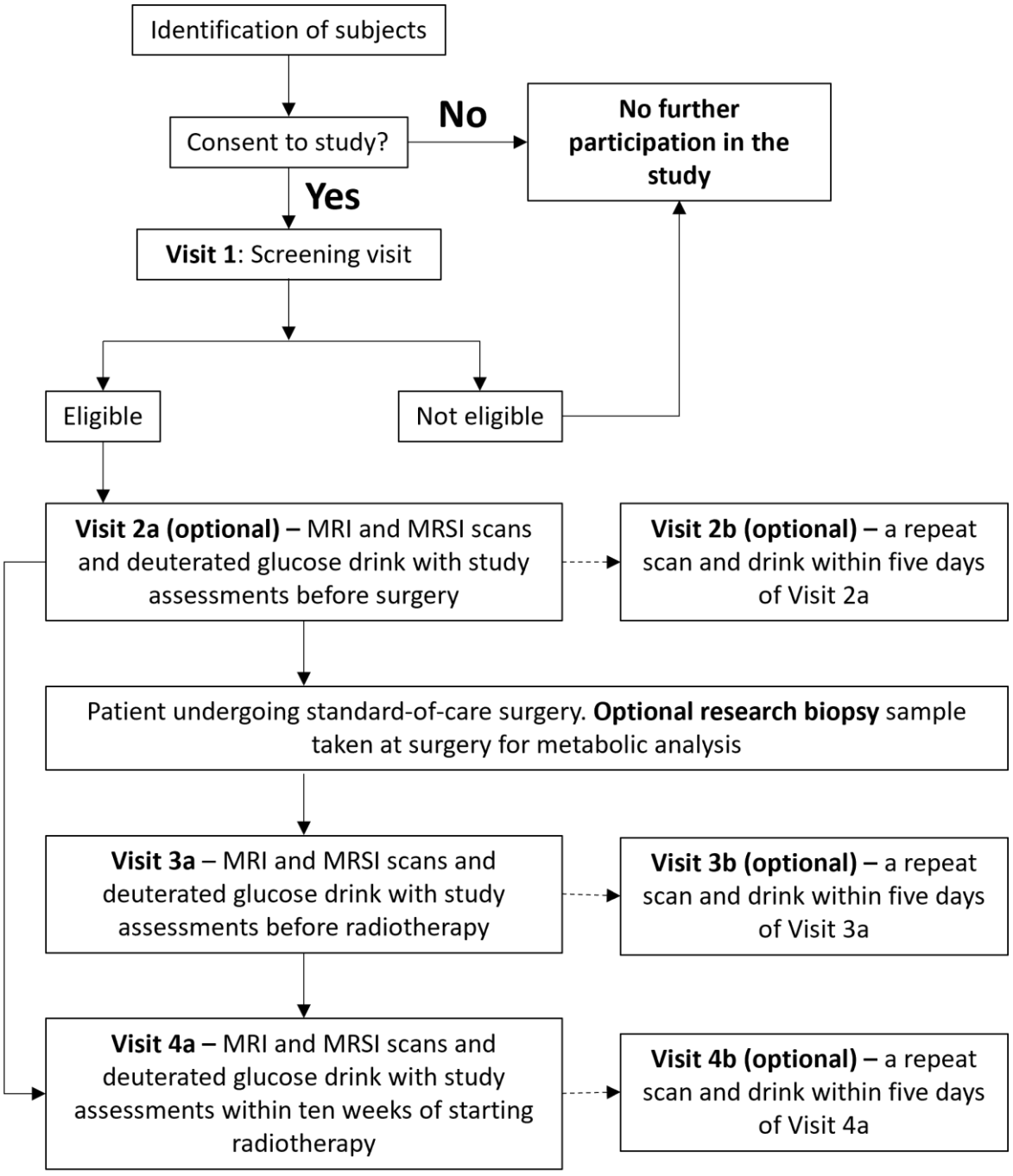
3 Table of Contents

4 Study Flow Chart

For Carbon-13 patients



For Deuterium patients



5 Investigators and Study Coordination

A list of sub-investigators and study coordinators, along with their start and completion dates on the project is retained in the Trial Master File.

6 Introduction

6.1 Background: hyperpolarised carbon-13 MRI

Development of new functional and molecular imaging techniques is necessary to improve patient care in the current era of molecular medicine and personalised care. Magnetic Resonance Spectroscopy (MRS) is capable of providing non-invasive detection of in vivo biochemical reactions and like Positron Emission Tomography (PET), can be used to investigate tumour cell metabolism. However, its lack of sensitivity results in production of images that are of relatively low spatial resolution. MRS also has relatively low temporal resolution, therefore limiting real-time imaging in humans and producing only a static picture of tissue metabolism.

Dynamic Nuclear Polarisation (DNP) or hyperpolarised Carbon 13 (^{13}C) Magnetic Resonance Imaging (MRI) is a new method that allows tissue metabolism to be studied non-invasively in vivo by increasing sensitivity to detection using MRS. Carbon-13 is a stable (i.e. non-radioactive) carbon isotope, which is Magnetic Resonance (MR)-active and detection of the ^{13}C-labelled metabolites formed from an injected ^{13}C-labelled substrate can provide information on metabolic fluxes. The technique of DNP involves placing ^{13}C-labelled compound in a magnetic field (3.35-5 Telsa (T)) at a temperature close to absolute zero (~1 Kelvin [K]) and microwave irradiation (1,2). In these conditions there is a significant increase in magnetic polarisation of the compound which, when the molecule is subsequently imaged using MRI, allows increase of the Signal-to-Noise Ratio (SNR) of between 10,000 and 100,000-fold (3). When hyperpolarised, the sample is rapidly warmed to body temperature and injected into the patient. The hyperpolarised molecule will pass through a number of safety checks in a Quality Control (QC) module before being injected into the study participant. The spatial distribution of the injected molecule can be imaged, as well as the metabolites formed from it, allowing surrogate maps of enzymatic activity to be calculated.

This novel technique has allowed dynamic imaging of cellular metabolic fluxes in vivo for the first time, with greatly improved signal to background noise ratios. The increased sensitivity is transient and following injection of a hyperpolarised molecule, the signal decays over a period of one to two minutes (the half-life is approximately 30 seconds in patients). We and others have undertaken studies to show the utility of hyperpolarised ^{13}C-pyruvate in humans (4,5). To enable this, we have created a bespoke cleanroom in Addenbrooke's Hospital Radiopharmacy and adapted the scanning facilities to allow for rapid injection and imaging of the hyperpolarised tracer.

6.2 Background: Deuterium Metabolic Imaging (DMI)

Deuterium (^{2}H), also known as heavy hydrogen is an isotope of hydrogen which contains one additional neutron within its nucleus compared to ordinary hydrogen (^{1}H), giving it double the mass of ^{1}H. Deuterium is found in low abundance in the body with 0.0115% of the natural abundance signal of ^{1}H. It can be detected using magnetic resonance spectroscopy and the SNR for detection is increased significantly after the administration of ^{2}H-labelled molecules. This can be either in the form of heavy water $^{2}H_2O$ or ^{2}H-labelled substrates. The latter includes ^{2}H labelled glucose and this has been termed deuterium metabolic imaging (DMI). Reliable differentiation between different metabolic states can be achieved when signals from ^{2}H-labelled glucose and its metabolites are detected in real time. DMI assesses the dynamics of isotope labelling on intermediate metabolites

after administration of isotope-labelled glucose or its breakdown products. Several recent studies have utilised DMI in vivo to study changes in brain metabolism (6,7,8,9). The studies confirm the ability of DMI to reliably measure spatiotemporal changes in cerebral glucose consumption rates and tricarboxyclic acid cycle flux though detection of ^{2}H-labelled glucose, glutamate/glutamine (Glx) and lactate in the brains of rats and humans both healthy volunteers and within brain tumours.

As part of this study we aim to demonstrate the feasibility of undertaking DMI and we also aim to co-recruit (where possible) the same participant to both substudies in DMI and carbon-13 MRI to see how they may provide complementary information.

6.3 Background: Chemical Exchange Saturation Transfer (CEST) imaging

Chemical Exchange Saturation Transfer (CEST) is an MRI-based technique which enables imaging of proteins, peptides and small molecules which are normally not detectable due to their low tissue concentration [61, 62]. The technique is based on selective saturation of solute protons in the molecule(s) of interest that resonate at frequency different from that of water by using a selective radiofrequency pulse. As the protons constantly move between the solute and the solvent pools in a process of chemical exchange, the saturation from solute pool protons is transferred to the bulk water pool. This causes reduction in the magnetization of water seen and reduction in water signal proportional to the concentration of the molecules of interest [61].

CEST can be used to detect of a number of endogenous or exogenous substances. The most studied application of CEST is amide proton transfer (APT)-CEST which is focused on a pool of molecules 3.5 ppm downfield from water, which correspond to slow-exchanging amides in proteins [62]. Nuclear Overhauser effect (NOE-CEST) arises from mobile macromolecules (-3.5 ppm), Amine-CEST from creatine and glutamate (2-3 ppm), and Gluco-CEST from exogenously administered glucose [61, 62].

APT-CEST has shown promising results in characterization of tumour microenvironment and metabolism in patients including brain tumours [61]. As a part of the second phase of DMI substudy (additina 11 patients) we propose to include an optional APT-CEST scan to provide pilot data on the relationship between the changes in tumour glucose metabolism (DMI) and mobile proteins and (APT-CEST).

6.4 Glioblastoma and pre-clinical studies

Glioblastoma (GB), also known as glioblastoma multiforme and grade IV astrocytoma, is the most common primary malignant brain tumour in adults, with a median survival of only 12-15 months despite aggressive therapy (10,11,12). This poor prognosis derives in part from the characteristic heterogeneity of this tumour, which results from a complex interplay between genomic and microenvironmental changes and is reflected in transcriptional and metabolic reprogramming (13). This metabolic reprogramming may influence whether a GB is predisposed towards infiltration or proliferation: highly proliferative non-migratory cells exhibit downregulation of glycolysis and upregulation of the pentose phosphate pathway (PPP) (14,15,16), whereas glioma stem cells are less glycolytic and have a higher mitochondrial reserve capacity, which has been correlated with resistance to radiotherapy (17). Metabolic reprogramming in GB therefore represents an important target for novel therapeutic strategies (12,14,20). Non-invasive methods for metabolic phenotyping of GB could help to better characterise tumours, as well as detect early response to treatment (14).

Pyruvate is a breakdown product of glucose and lies at a metabolic crossroads, between conversion to lactate in the reaction catalysed by cytosolic lactate dehydrogenase (LDH), and entry into the mitochondrial tricarboxylic acid (TCA) cycle in the reaction catalysed by pyruvate dehydrogenase (PDH). The latter transfers the pyruvate ^{13}C label to carbon dioxide, which is in near-equilibrium with bicarbonate. The bicarbonate signal is detected due to its greater abundance at physiological pH

(22,24). Preclinical studies have shown increased lactate labelling in orthotopic GB models and have demonstrated changes in lactate labelling following therapy (24,25,26,27,28). EGFR amplification in GB results in increased levels of the transcription factor c-Myc, which increases glycolytic flux (14). A recent study in orthotopically implanted patient-derived xenograft models of GB demonstrated a high degree of variability in lactate labelling between the models, which could be explained by differences in the levels of c-Myc driven LDH-A expression and glycolytic activity (29). ^{2}H-labelled glucose can also be used to probe both glycolytic formation of lactate as well as glutamine/glutamate through glucose oxidation.

6.5 Data from clinical studies

The first-in-human metabolic imaging study using hyperpolarised ^{13}C-pyruvate was performed at the University of California, San Francisco (UCSF) (5). This study evaluated the safety and feasibility of hyperpolarised ^{13}C-pyruvate as an agent for non-invasively characterising alterations in tumour metabolism for patients with prostate cancer. The study demonstrated both the safety of the agent and also elevated ^{13}C lactate/^{13}C-pyruvate in regions of biopsy-proven cancer. We have injected 90 participants (up to 30th November 2020) with hyperpolarised ^{13}C pyruvate in Cambridge with no adverse events observed. We have demonstrated the formation of lactate in both the normal brain and in tumours (breast, glioma, renal cancer), as well as intratumoral and intertumoral heterogeneity in the formation of lactate. We have preliminary data showing changes in lactate following treatment. Intravenous hyperpolarised [1-^{13}C]-pyruvate has been shown to result in both lactate and bicarbonate labelling in the normal human brain, allowing assessment of both glycolytic metabolism in the cytosol and oxidative metabolism in the mitochondria (30). Previous reports have demonstrated the feasibility of probing the metabolism of GB and brain metastases using the technique (31,32,33,34,35,36). We have recently investigated hyperpolarised [1-^{13}C]-pyruvate metabolism in seven treatment-naïve IDH wildtype GB patients showing metabolic heterogeneity. Here we will extend this work to include the effects of standard of care treatment on metabolism.

Recently De Feyter et al (6) translated DMI to human studies, demonstrating lactate and glutamine/glutamate formation in normal brain and lactate labelling in GBM.

There is increasing evidence on the usefulness of APT-CEST in glioma [62, 63]. As in high grade glioma the concentration of mobile proteins and peptides is increased compared to surrounding tissue, the resulting APT-CEST signal has been used to characterize tumour extent and grading [62]. Interestingly, APT-CEST signal may extend beyond contrast-enhancing tumour, which is usually considered to be the aggressive part of the neoplasm that is normally targeted for surgical resection, and may suggest the presence of higher grade component beyond the boundary of contrast enhancement. Similarly, in tumours appearing to be of low grade presence of APT-CEST signal may indicate the presence of higher grade component (63). These differences in the intratumoural contrast are not fully understood but point to application of CEST in planning of surgical resection extent and/or radiotherapy treatments [62, 63]. Currently there is no data on how APT-CEST signal relates to altered glucose metabolism in the tumour.

6.6 Cambridge SPINLab

The procedure involved in ^{13}C DNP has been refined since its inception and GE Healthcare has developed commercial equipment called SPINLab that carries out this process. There are a number of sites around the world that have SPINLabs installed which are currently being used with other molecules, such as ^{13}C-pyruvate. The University of Cambridge installed one of the world's first SPINLabs at the Cambridge Biomedical Campus in 2013 and has achieved promising results from pyruvate work. A further SPINLab was installed in 2016 within another location at the Cambridge Biomedical Campus to allow for the possibility of hyperpolarised PET/MR imaging. These devices include quality control checks to ensure safety for human use and we have successfully polarised hundreds of samples for hyperpolarised ^{13}C-pyruvate at Cambridge.

6.6.1 The GE Healthcare SPINLab Pharmacy kit

A special pharmacy kit has been designed by GE Healthcare for use with the SPINLab when hyperpolarising samples intended for imaging participants. The kit is a single use, disposable unit and contains all the components required to hyperpolarise a sample, dissolve it into an injectable form and carry out quality control checks. The kit is assembled in a sterile environment in a dedicated aseptic unit. Appropriate facilities to permit work with the pharmacy kits have been installed at Cambridge. During assembly for use with pyruvate, the sample vial is filled with a mixture of the ^{13}C-labelled molecule to be hyperpolarised and a chemical termed EPA (electron paramagnetic agent). EPA is a chemical which supplies electrons from which spins are transferred to the ^{13}C nuclei of the target compound resulting in its hyperpolarisation. After filling the sample vial it is sealed with a laser welder and the setup is placed in the SPINLab for hyperpolarisation.

6.6.2 Human imaging with ^{13}C-pyruvate

In this study we propose to address the question of whether ^{13}C-pyruvate metabolism can be imaged in humans using this technique. We will assess ^{13}C-pyruvate signal in participants with brain tumours and will undertake MRI and MRSI before surgery and/or before and after standard of care therapy. We will then investigate if the therapy changes ^{13}C-pyruvate metabolism and if this can be used to predict treatment response.

The metabolism of ^{13}C-pyruvate could improve diagnostic assessment of cellular metabolism in tumours and could facilitate selection of the best personalised treatment regimens.

7 Rationale for Study

The aim is to develop a technical setup for metabolic brain scanning and following implementation to quantify the efficacy of tumour therapy in brain tumour patients based on the recently invented MR-hyperpolarisation scanning technique. This imaging study will: 1) acquire dynamic data from human tissues following the injection of hyperpolarised ^{13}C-pyruvate and use ^{13}C-MRI to monitor spatial and temporal changes in the ratio of ^{13}C-lactate to ^{13}C-pyruvate and/or 2) acquire dynamic DMI data from human tissues following the oral consumable of deuterated glucose, and (in the second phase of the study) optional APT-CEST pilot imaging data. Data acquired during this physiological study will be used to optimise future imaging protocols.

8 Study Design

8.1 Overview

8.1.1 Statement of design

This is a non-randomised, physiological imaging study split into two substudies; 1) using dynamic ^{13}C-MRI and MRSI in up to 11 brain tumour patients and 2) using dynamic deuterium metabolic MRI and MRSI imaging (DMI) in up to 22 brain tumour patients (including optional APT-CEST imaging for up to 11 patients). Where possible, we would aim to co-consent the same patient to both substudies which will involve performing both MRI scans at the same imaging visit, however, these may not be possible in some instances and therefore, patients will be to be recruited to one of the substudies and not the other.

If the patient is selected for surgery, they will be offered an **optional** MRI scan before their surgery and also an **optional** research biopsy sample taken at their surgery. If the patient is not selected for surgery or those that have had surgery go on to receive radiotherapy, they will be offered an MRI scan before

commencement of radiotherapy and within ten weeks of starting radiotherapy. In order to be classed as evaluable, the patient will need to complete the before commencement of radiotherapy scan **AND** the scan within ten weeks following commencement of radiotherapy. If the patient does not complete both scans (either due to patient withdrawal or researcher decided withdrawal), they will be replaced by another patient.

This study will not change the treatment that has been determined for the patients either as part of their standard of care or another study.

Patients will not receive more than four injections of ^{13}C-pyruvate and will not receive more than four deuterated glucose drinks.

8.1.2 Number of centres

This single centre study is being conducted at Addenbrooke's Hospital, Cambridge Biomedical Campus, UK. As part of the overall project, we are collaborating with Aarhus University, Denmark who are undertaking a similar single centre study in parallel.

8.1.3 Number of subjects

Up to 11 patients with brain tumours will be scanned under the ^{13}C-MRI substudy and up to 22 patients with brain tumours will be scanned under the DMI-MRI substudy (with up to 11 patients also undergoing an optional APT-CEST acquisition). Where possible, these will be co-consented to both substudies.

8.1.4 Study duration

The expected duration of the study is up to ten years. Each patient will be actively enrolled into the study for a period of up to nine months from consent to the study. A follow up for disease progression/mortality through their clinic appointments or GP may be required for up to 36 months after the final injection/drink.

8.1.5 Study objectives

Primary objective(s):

For the Hyperpolarised MRI substudy: Detection of hyperpolarised ^{13}C-pyruvate and its metabolite ^{13}C-lactate using dynamic MRI and MRSI to study pyruvate metabolism in normal tissue and in tumours.

For the DMI MRI substudy: Detection of ^{2}H-glucose and its metabolites (lactate and glutamine/glutamate) using dynamic MRI and MRSI to study metabolism in normal tissue and in tumours. To compare distribution of glucose and its metabolites with distribution of protein pool measured by APT-CEST.

For both substudies: To quantify whether the MRI techniques can identify tumour response to standard-of-care radiotherapy +/- chemotherapy (or in the case of experimental radiotherapy +/- chemotherapy where this study would run in parallel to another ethically-approved study).

Secondary objective:

To examine whether levels of lactate normalise within the normal appearing brain following therapy.

Where possible, to examine correlations between tissue expression (archival or fresh tissue) of metabolic and other markers (such as LDH) and the MR signal acquired from the dynamic ^{13}C-lactate/^{13}C-pyruvate MRI data.

8.2 Selection of subjects

Patients with suitable cancers will be identified through multidisciplinary team (MDT) meetings or by clinical teams involved in their routine care at Cambridge University Hospitals NHS Foundation Trust. Participants will be eligible for the imaging study if they meet all the inclusion and none of the exclusion criteria as detailed below.

8.2.1 Inclusion criteria

- Over 18 years old
- Able to and provide written informed consent to participate
- If female, postmenopausal or if women of childbearing potential (WOCBP) using a suitable contraception
- If male, using a suitable contraceptive method for the duration of the study

8.2.2 Exclusion criteria

The presence of any of the following will preclude participation as determined by the delegated investigator:

- Contraindication or inability to tolerate MRI
- Pregnant or actively breast-feeding woman
- If using an intrauterine contraceptive device (IUCD) as a method of contraception the device should be MRI safe at 3 T (researcher to confirm)
- High blood glucose level (as determined by the researcher) that may have an impact on the study results
- Significant medical or psychiatric history rendering the subject ineligible as deemed by the investigators

8.2.3 Subject withdrawal criteria

Participants may be removed from the study at their choice or at the Investigator's discretion if it is felt to be clinically appropriate. Reasons for participant withdrawal will be recorded. Primary reasons for withdrawal may include: Serious Adverse Event (SAE), withdrawal of consent, lost to follow up, participant non-compliance, or study closed or terminated. Participants who are withdrawn from the study or do not complete the pre- and post-therapy scans will be replaced.

8.2.4 Study restrictions

Women of childbearing potential are required to use adequate contraception for two weeks before and two weeks after each imaging visit. This includes:

- Intrauterine contraceptive device
- Vaginal ring
- Hormonal based contraception (pill, contraceptive injection, etc.)
- Adequate barrier contraception
- True abstinence

Men must use adequate barrier contraception (such as condoms) for two weeks after each imaging visit as a precaution.

8.3 Study plan

Study participants will be deemed evaluable if they receive ^{13}C-pyruvate injections and/or deuterated glucose drink at the pre- and post-therapy timepoints. Each study participant will be allocated a unique study number following study enrolment and will be identified by this number throughout the data collection and analysis process.

The participants will be asked to attend all or some of these timepoints:

1. **Optional** imaging visit before surgery with **optional** repeat imaging visit within five days of this scan (where possible).
2. Standard of care surgery with **optional** research biopsy taken at surgery for metabolic analysis.
3. **Mandatory** imaging visit before radiotherapy with **optional** repeat imaging visit within five days of this scan.
4. Imaging visit within ten weeks of starting radiotherapy with **optional** repeat imaging visit within five days if the patient remains well enough to continue with the study.

The definition of **mandatory** above is that the participant should be willing to undertake at least the baseline timepoint. If during the study, they decide to withdraw their consent and not take part in the second mandatory scan they are free to do without any impact on their standard of care.
If the researchers determine during the study that the participant is no longer suitable, this would mean the participant may not take part in the second mandatory scan. If a participant does not complete both mandatory scans, they will be replaced.

No participant will receive more than **four injections of ^{13}C-pyruvate or four deuterated glucose drinks in total**. This may mean that some patients will not be asked to perform all timepoints above. This will be determined by the researchers and discussed with the patient at initial consent to the study. Up to 11 participants who receive DMI scan may receive APT-CEST.

8.4 Procedures and assessments

All the procedures will be performed at the Cambridge Biomedical Campus.

8.4.1 Recruitment and informed consent

A copy of the Participant Information Sheet will be given to each potential participant and the participant will be allowed a minimum of 24 hours to consider this information. If they decide to take part, they will sign the informed consent form prior to any protocol specific procedures. Where possible, participants will be consented and scanned for the study during their standard of care visits to the hospital. Consenting and screening of participants will be undertaken by members of the study team who are on the delegation log.

8.4.2 Visit 1: Consenting and clinical assessment before the first imaging round

The schedule of assessments is detailed in Section 8.4.7. The following procedures and assessments will take place:

- Informed consent
- Medical history using the patient's medical records and clinical examination (heart and lung auscultation)
- Sex, height, weight, age and date of birth
- Heart rate, BP, pulse oximeter reading

This will be undertaken before the first imaging visit (visit 2a or 3a) but may form part of the imaging visit to reduce the number of visits required for participant.

8.4.3 Imaging visits

Timepoints for scanning visits are shown in Section 8.3. Subjects may be requested to fast for six hours before imaging e.g. missing breakfast if imaged in the morning or lunch if imaged in the afternoon after their verbal consent to participate in the study. There may be specific reasons why this is not possible for an individual patient and researchers will factor this in before consenting the patient to the study.

A maximum of 50 mL of blood will be collected for this study from the participant with a cannula at each visit. To check participants glucose levels prior to scanning, we may require a glucose measurement to be taken using a standard finger prick method or as detailed below using a commercially available glucose monitor.

At imaging visits that require an injection of ^{13}C-pyruvate or deuterated glucose drink, the following observations, measurements and procedure may be undertaken if they have not already been undertaken as part of routine clinical assessment or the initial study assessment:

- Heart rate, blood pressure, pulse oximeter reading pre- and post-imaging
- Any clinically directed assessment
- Pre-imaging creatinine (if clinically indicated; venous blood sample or rapid test) and blood glucose test (finger prick test or point of measurement where possible)
- Pre-imaging pregnancy test for WOCBP
- ^{13}C-pyruvate injection(s) and/or deuterated glucose drink
- Venous blood samples before and after imaging for research purposes, where possible

In up to 11 patients (of DMI substudy) an optional APT-CEST MRI scan will be obtained prior to DMI acquisition. The scan uses a standard head coil with no contrast injection and only adds 7 minutes imaging time. As the DMI scan includes delay to allow absorption of the orally administered glucose, the additional APT-CEST scan will not extend the duration of the imaging visit, and poses minimal burden to the patient.

8.4.4 Optional biopsies

If the participant agrees to the **optional** before surgery imaging visit (see Section 8.3), they will be asked if they are willing to have **optional** research biopsies of their tumour during their standard of care surgery. The patients will be able to opt out of this and will not affect their enrolment in the study.

Analysis of these samples will be performed suitably anonymised and will be investigating lactate and gene expressions for metabolic related proteins and will be correlated to the imaging. Analysis may involve samples being sent to a third party for analysis, but this will be anonymised. These samples will

either be destroyed once analysis has been completed or returned to the research group when analysis is complete.

8.4.5 End of study participation

The end of the active study phase will be up to nine months after the first imaging visit, however, researchers may follow up with the clinical team or GP for disease progression/mortality for up to 36 months after the final injection/drink. No patient is expected to be on the study longer than three years after enrolment.

8.4.6 Long-term follow-up assessments

No long-term follow-up over the duration of the study is planned.

8.4.7 Schedule of assessments

Assessment	**Visit/Days**	
	Visit 1: Clinical examination visit before the first imaging round[a]	**MRI and injection visit(s)**
Attend Unit	*	*
Consent [b]	*	*
Medical History	*	
Demography (weight, height, sex, Date of Birth)	*	
Clinical Examination	*	
ECOG performance score	*	
Renal function test [c] (creatinine value)	* as clinically indicated	* as clinically indicated
Pre-imaging blood glucose test [d]		*
Venous blood sample [d] - Before imaging - After imaging		 * *
Pregnancy test in WOCBP		*
MRI scan(s) (± a sequence with gadolinium)		*
General/additional assessments		* as clinically indicated
Vital Signs (Heart rate, BP, pulse oximeter reading) - Pre-imaging - Post-imaging	*	 * *
Injection of ^{13}C-pyruvate and/or deuterated glucose drink		*

* Applicable assessment

a. We will attempt to screen for patients during their standard of care visits and use of medical records to collect clinical data relevant to the study

b. Ongoing consent will be verbally confirmed at each visit

c. Either recent (< 2 weeks) venous blood sample or point of care test as clinically indicated

d. Pre-scan blood glucose test (finger prick test or point of care test where possible)

e. Venous blood samples for research purposes, where possible

8.4.8 Blood sampling for laboratory analysis

Blood samples will be taken by the research team for research purposes and analysed e.g. or haematological and biochemical parameters, which will include but not limited to full blood count, glucose, lactate, lactate dehydrogenase and other biochemical measurements. These samples will either be analysed in the Department of Pathology at Cambridge University Hospitals NHS Foundation Trust or within dedicated laboratory space as part of the University of Cambridge. Total blood volumes for the study are planned to be in accordance with National Institute of Health Research guidelines that states that less than 550 mL blood should be collected per eight week period.

9 Imaging Agent Details

9.1 Route of administration and maximum dosage allowed: ^{13}C-pyruvate

Single intravenous injection of up to 40 mL a 0.4 mL/kg of ~250 mM ^{13}C-pyruvate. There will be an **optional** repeat imaging injection of ^{13}C-pyruvate at all imaging visits to test for repeatability of the ^{13}C-MRI technique. These will take place within five days of the first scan.

9.2 Route of administration and maximum dosage allowed: deuterated glucose drink

At each imaging visit the participant will receive a deuterated glucose solution where 60 g of glucose is dissolved in 200 mL of potable water and the dose solution will be adjusted to their body weight at 0.75 g/kg body weight.

9.3 Maximum duration of investigations

We will endeavour to minimise the number of injections and/or glucose drinks for each participant, however, the maximum number of injections/drinks will be limited to four each.

9.4 Hyperpolarised ^{13}C-pyruvate

In this study, ^{13}C-pyruvate will be hyperpolarised using a clinical hyperpolariser (SPINLab), which involves cooling the molecule to a very low temperature in a magnetic field where the nuclei align; this is then rapidly warmed to approximately body temperature before being injected intravenously into a participant. After being warmed, the hyperpolarised pyruvate will pass through a number of safety checks before being injected into the study participant. This will be injected through a cannula while they are laid on the scanner bed.

Hyperpolarisation does not change the chemical properties of ^{13}C-pyruvate and hence should not modify its effects on body chemistry. ^{13}C-pyruvate is a non-radioactive form of pyruvate and ^{13}C labelled molecules are present in the body at 1.1% natural abundance.

9.5 Deuterated glucose

The deuterated glucose molecules are manufactured and stored in vials that are suitable for single doses only and will be mixed immediately before consumption with potable water for the participant to drink.

Deuterated molecules (such as deuterated water) are present within the body at 0.0115% natural abundance.

10 Assessment of Safety

Definitions are presented in Appendix 2.

10.1 Adverse Reactions/ Expected Adverse Events

There are no expected adverse reactions (AR) associated with ^{13}C-pyruvate and deuterated glucose MRI. If any AR's are observed during this study, they will be recorded on the proforma and reviewed by the research team.

The following adverse events (AE) are known side effects of the assessment procedures:

- Bruising at the sites of venopuncture
- For those patients having the ^{13}C-pyruvate injection, transient local reaction at site of injection (mild flush and signs of erythema)

They are generally not serious in nature and will not be recorded in the AE/AR log as part of this study.

Participants with solid malignancies are expected to have cancer and treatment related adverse events and some of them may be Serious Adverse Events (SAE) (according to the definition in Appendix 2). However, as these are related to cancer rather than the study procedures they will not be recorded or collected as study data during this study. Only study procedure related SAE will be recorded.

10.2 Recording, evaluation and reporting of adverse events

The Sponsor expects that all adverse events are recorded from the point of Informed Consent.

All Adverse Events and Adverse Reactions will be assessed by the Investigator and recorded in medical notes as well as on the proforma (with the exception of expected AEs and SAEs related to cancer).

Individual adverse events should be evaluated by the Investigators. This includes the evaluation of its seriousness, causality, severity and any relationship between the medicinal product(s) and/or concomitant therapy and the adverse event (see definitions in Appendix 2).

The Chief Investigator is responsible for the prompt notification to the Sponsor and the Research Ethics Committee that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was:

- 'related': that is, it resulted from administration of any of the research procedures; and
- 'unexpected': that is, the nature and severity of the event is not listed in the protocol or the investigators brochure as an expected occurrence.

Reports of related and unexpected SAEs should be submitted to the Sponsor and the Research Ethics Committee (REC) within 15 days of the Chief Investigator becoming aware of the event.

11 Toxicity – Emergency Procedures

No toxicity is expected as pyruvate or glucose as they are endogenous products. However, in the event of an acute hypersensitivity reaction, supportive care will be given to the participant according to local clinical procedures.

12 Data and Study Management

12.1 Evaluation of results (definitions and response/evaluation of endpoints)

For scanning performed in the Department of Magnetic Resonance Imaging and Spectroscopy (MRIS Unit), the MRI will be conducted and records will be kept electronically on Picture Archiving and Communication System (PACS) for further analysis. Analysis of the data will be performed on anonymised images and be conducted as required.

For scanning performed in the Wolfson Brain Imaging Centre (WBIC), the MRI will be conducted and records will be kept on a non-anonymised but encrypted server. Analysis of the data will be performed on anonymised images and be conducted as required.

12.2 Statistics

12.2.1 Statistical methods

Descriptive statistics will be used. The primary covariates to be studied will be as follows:

- For the ^{13}C-MRI experiments: Ratio of the summed hyperpolarised ^{13}C-lactate over summed ^{13}C-pyruvate over the timecourse of the experiment as a quantitative metric of pyruvate to lactate exchange catalysed by the enzyme lactate dehydrogenase. We have extensive experience in developing quantitative methodology to analyse this data (59).
- For the DMI-MRI: Ratio of the summed ^{2}H-lactate over summed ^{2}H-glutamine/glutamate as a measure of the ratio of glycolysis to oxidative metabolism.
- For the APT-CEST: APT-CEST signal calculated as reported by Warnert (63).

The study has been powered to assess changes in the ^{13}C-pyruvate metabolism and we have data on 7 GBM patients (publication in submission). This work showed the tumour lactate/pyruvate ratio was 0.34±0.06. We do not have preliminary evidence of the effect of treatment response in GBM but in other on-going work we have demonstrated that a >20% change in this lactate/pyruvate ratio was predictive of complete pathological response in breast cancer patients undergoing neoadjuvant chemotherapy (publication in submission). We have therefore assumed that we will also identify a minimum 30% change in GBM as part of this study (i.e. a change in the lactate/pyruvate ratio of 0.10). Approximately 11 (10.8) patients are required to identify this 30% change, assuming 80% power and 90% significance (60). As there is no equivalent data available for the DMI MRI in GBM, a similar number of patients will be co-recruited (where possible) as part of this experimental arm of the study. Further 11 patients may be recruited to increase the statistical power and strengthen the reliability of the final results.

12.2.2 Number of subjects to be enrolled

We plan to include up to 11 patients with brain tumours in the ^{13}C pyruvate substudy and up to 22 patients with brain tumours in the DMI substudy (including 11 patients for APT-CEST scan). Where possible, we will aim to co-consent the same patient to both substudies.

12.2.3 Criteria for the premature termination of the study

If significant toxicity is observed directly related to the procedure, a careful review of the data will be performed and a decision on subsequent dosing, including the possibility of prematurely terminating the study, will be undertaken.

If no pyruvate, lactate or deuterated signal is detected in any of the participants, the study will be re-evaluated by the investigators.

12.2.4 Definition of the end of the study

The study will end when the last participant has attended the last assessment or follow-up visit or when the Chief Investigator determines that the study should be closed.

12.3 Study management responsibilities for data handling and record keeping

12.3.1 Proforma

All data collected during the study will be collected or transferred into a proforma. An electronic proforma may be used if relevant. The proforma will be accessible to relevant study team members.

12.3.2 Data protection and patient confidentiality

All investigators and study site staff involved in this study must comply with the requirements of the General Data Protection Regulation (GDPR) 2018 and Trust Policy with regards to the collection, storage, processing and disclosure of personal information and will uphold core principles. The personal data recorded on all documents will be regarded as strictly confidential.

12.3.3 Preparation and submission of amendments

The Radiology Research Team will be responsible for the day-to-day running of the study, for preparing all essential documents and amendments to these and for submitting Annual Progress Reports to the REC. These annual reports will be reviewed by the Sponsor prior to submission. Copies will be filed in the Study File.

12.3.4 Study documentation and archiving

It is the responsibility of the Chief Investigator to maintain the Study File.

All essential source and study documentation (e.g. Study File, source data, proforma) will be securely archived after the last analysis of the study data has been completed and the Final Study Report has been submitted to the relevant authorities. Archiving must be provided as per local policy or the length of time specified by current applicable legislation, whichever is the longer.

The Investigator must not destroy any documents or records associated with the study without written approval from the Sponsor.

12.4 Research Steering Group

A Research Steering Group (RSG) will oversee the project according to the Grant agreement with the funder.

13 Ethical and Regulatory Considerations

13.1 Consent

The Informed Consent form must be approved by the REC and must be in compliance with GCP, local regulatory requirements and legal requirements. The investigator must ensure that each study participant, or his/her legally acceptable representative, is fully informed about the nature and objectives of the study and possible risks associated with their participation.

The Chief Investigator (or suitably trained delegate) will obtain written informed consent from each participant before any study-specific activity is performed. The informed consent form used for this study and any change made during the course of this study, must be prospectively approved by the REC. The investigator will retain the original of each signed informed consent form.

Should a participant require a verbal translation of the study documentation, it is the responsibility of the research team to use a local, Trust approved translator service.

13.2 Ethical committee review

Before the start of the study or implementation of any amendment we will obtain approval of the study protocol, protocol amendments, informed consent forms and other relevant documents e.g., advertisements and GP information letters if applicable from the REC. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

13.3 Regulatory issues

This study is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the European Union (EU) Directive 2001/20/EC and no submission to the Clinical Trials Unit at the Medicines and Healthcare products Regulatory Agency (MHRA) is required.

13.4 Protocol amendments

Protocol amendments must be reviewed and agreed by the Sponsor prior to submission to the REC.

13.5 Declaration of Helsinki and Good Clinical Practice

The study will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of GCP, the protocol and applicable local regulatory requirements and laws.

14 Sponsorship, Financial and Insurance

The study will be jointly sponsored by Cambridge University Hospitals NHS Foundation Trust and University of Cambridge. Funding for this project comes from The Lundbeck Foundation, Denmark.

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical study caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the study, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising thorough participation in the clinical study.

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Appendix 1 ECOG Performance Status

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

ECOG PERFORMANCE STATUS

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

* As published in *Am. J. Clin. Oncol.* [16], credit to the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix 2 Definition for Safety Assessment

Adverse event (AE)

Any untoward medical occurrence in a patient or clinical study subject administered a product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Please note: Recording of all adverse events must start from the point of Informed Consent regardless of whether a patient has yet received a medicinal product (GCP requirement).

Adverse reaction (AR)

All untoward and unintended responses to a product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected adverse reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product).

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.

Serious adverse event or serious adverse reaction (SAE or SAR)

Any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect,
- or is otherwise considered medically significant by the Investigator.

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

Important medical events should be reported on the serious adverse event (SAE) form under the ‘other’ category.

Individual adverse events should be evaluated by the investigator. This includes the evaluation of its seriousness, causality, severity and any relationship between the medicinal product(s) and/or concomitant therapy and the adverse event.

Assessment of seriousness

Seriousness is assessed against the criteria for SAE/SAR. This defines whether the event is an adverse event, serious adverse event or a serious adverse reaction

Assessment of causality

Definitely: A causal relationship is clinically/biologically certain. This is therefore an Adverse Reaction

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the medicinal product and there is a reasonable response on withdrawal. This is therefore an Adverse Reaction.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the medicinal product. This is therefore an Adverse Reaction.

Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible. This is therefore an Adverse Event.

Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible. This is therefore an Adverse Event.

Clinical assessment of severity

Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated

Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity

Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the subject's life is at risk from the event.