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STUDY PROTOCOL

A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy

COMPLIANCE

This study will be conducted in accordance with the principles of Good Clinical Practice (GCP). It will also be conducted in compliance with the protocol, Data Protection Act and other regulatory requirements, as appropriate.

AUTHORISATION

Professor Paul Abel (Chief Investigator) is authorised to sign the final protocol and protocol amendments:

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LIST OF ABBREVIATIONS

ADL	Activities of daily living
ADT	Androgen deprivation therapy
AE	Adverse event
CANTAB	Cambridge neuropsychological test automated battery
EDTA	Ethylenediamine tetracetic acid
FBC	Full blood count
FBP	Filtered back projection
fMRI	Functional magnetic resonance imaging
ICHT	Imperial College Healthcare NHS trust
KDa	Kilo-dalton
LHRHa	Luteinising hormone releasing hormone agonists
LVLS	Last visit of the last subject
mBq	Millibecquerel
MCI	Mild cognitive impairment
mM	Millimolar
MRI	Magnetic resonance imaging
OSEM	Ordered subset expectation maximisation
PET	Positron emission tomography
PIS	Patient information sheet
ROI	Region of interest
SAE	Serious adverse event
SHBG	Sex hormone-binding globulin
SPM	Statistical parametric mapping
SUV	Standardised uptake value
TSPO	Translocator protein
VT	Total volume of distribution

STUDY DESIGN

To compare quantitative [^{11}C]PBR28 positron emission tomography (PET) imaging in patients with clinically significant androgen deprivation therapy (ADT) associated mild cognitive impairment (MCI) with an age and treatment matched cohort with no MCI.

Six patients reporting and assessed to have significant ADT related MCI and who are subsequently shown to have the genetic polymorphism demonstrating high [11C]PBR28 binding will be scanned. Comparison of these data will be made with scans of 6 age and treatment matched patients with no evidence of MCI who again are shown to express high affinity 18 kDa TSPO/Peripheral benzodiazepine receptors.

MCI will be assessed by neuropsychological and cognitive assessments (~~standard~~ paper and computer-based~~) questionnaires and online)~~ and its neural basis will be investigated using magnetic resonance imaging (MRI).

SCIENTIFIC RATIONALE

In males, MCI with aging is thought in part to be related to reduction in serum androgen level and international studies are on-going to prevent age-related cognitive decline using androgen replacement therapy. Reduction in cognitive function often leads to morbidity and reduction in quality of life. The commonest therapeutically induced reduction in sex hormone level in men is in the treatment of prostate cancer. As prostate cancer is androgen dependent for growth, androgen-deprivation therapy (ADT) to suppress serum testosterone level to castration levels (< 1.7mM) is the key therapeutic intervention for advanced disease. Up to 1 million men worldwide are estimated to have been prescribed ADT for prostate cancer, mostly using luteinising hormone releasing hormone agonists (LHRHa). ADT is now also used to treat some early prostate cancer and as early asymptomatic prostate cancer is increasingly being diagnosed and treated following screening with serum PSA measurement, estimates suggest that eventually up to 4% of all Caucasians will be castrated, some of them remaining on ADT for as long as 10 years or more. ADT is associated with considerable adverse effects, including MCI. Up to 69% of men showed MCI after six to nine months of ADT, with a decline in at least one cognitive area, most commonly visuospatial ability and executive function. Little work has been done to quantify MCI due to ADT, understand the mechanism, predict which patients will be affected and determine ways of reducing this side effect.

Establishing the presence of pathophysiology in ADT induced MCI first is important due to the lack of understanding of the underlying mechanism. A plausible scientific approach in this regard appears to be the use of the brain's immunological response to pathology using a PET imaging ligand for activated microglia. Brain microglia have been demonstrated to be highly responsive to brain injury and are rapidly activated in an attempt to envelope/ contain the focal pathology. When activated, brain microglia have been shown to express the translocator protein (TSPO). The 18KDa translocator protein (TSPO) formerly known as the peripheral benzodiazepine receptor (PBR) is widely expressed in the body but is particularly enriched up to 20 to 50 fold in steroid synthesising tissues. High TSPO expression has also been reported in immune cells such as macrophages and monocytes and TSPO is a well-characterised marker of neuroinflammation. PET imaging ligands have been developed for TSPO and used successfully for researching a range of brain disorders. While such imaging does not provide mechanistic information of the underlying pathology,

except for the exacerbation of frank neuro-inflammation, it does offer a generic sensitive bio-marker for demonstrating the presence of an on-going active pathology.

OBJECTIVES OF THE STUDY

This is a pilot study to determine if there is an increase in uptake of the [11C]PBR28 PET biomarker in patients who have been clinically assessed to have ADT induced MCI.

Primary Objective

- To demonstrate, using a PET imaging bio marker of activated microglia (TSPO), the presence of regional or global pathological changes in the brain that relate to hormonal cancer therapy induced MCI.

Secondary Objectives

- To provide insight into possible mechanisms of hormonal therapy induced MCI i.e. association of cognitive and neuropsychiatric impairments following ADT with structural and/or functional brain connectivity.
- To design and provide a power calculation for a subsequent comprehensive and mechanistic based molecular imaging study of hormonal therapy induced MCI.

OUTCOME MEASURES

The outcome measure is the increased uptake of [^{11}C]PBR28 biomarker by activated microglia of patients demonstrating significant MCI with the primary endpoint of [^{11}C]PBR28 uptake measured as a standardised uptake value (SUV). ~~and secondary endpoint as the [^{11}C]PBR28 total volume of distribution (VT).~~

PATIENT RECRUITMENT

Patients will be recruited from the uro-oncology follow-up clinics of Imperial College Healthcare NHS trust (ICHT). The potential participants will be identified and approached in the clinic by their treating consultants. The consultants will then refer interested patients to a member of the research team to give details of the study.

Before a patient is entered into the study, written informed consent will be obtained. When obtaining consent from a patient, the study and the patient information sheet (PIS) will be introduced in full by a member of the research team and then consent taken. Patients will be allowed at least 24 hours to consider participation but they may take as long as they wish before making a decision.

The participants will be asked about any previous radiotherapy. Their medical notes will be reviewed for the same purpose and in the case of any concern, a direct check will be made with the radiotherapy department. Participants will be asked about any scans they have had in the last year and their medical notes and computerised radiology record will also be reviewed for the same.

Inclusion Criteria

1. Prostate cancer patients between the ages 50 to 80 years on ADT with LHRHa for at least 3 months and up to a year.
2. Able to give written informed consent.
3. ~~Suitable for insertion of an arterial line.~~
4. ~~3.~~ Able to lie still for up to 90 minutes for a PET scan.

~~5.~~4. Not claustrophobic and so able to undergo an MRI scan.

Exclusion Criteria

1. Patients with a known history of organic brain disorders and associated dementia, delirium and other specific neuropsychiatric conditions, including stroke and head injury.

~~2. Patients with a known history of Raynaud's disease, thromboangiitis obliterans.~~

~~3.~~2. Patients who are clinically assessed as having MCI prior to starting ADT.

~~4.~~3. Patients with a medical prognosis of less than 3 months survival.

~~5.~~4. Patients who are claustrophobic.

~~6.~~5. Patients who have any metal implanted in their body e.g. heart pacemaker, cochlear implant or any other electronic device.

Sample size

A total of 12 prostate cancer patients on ADT with LHRHa will be recruited for the imaging~~PET scanning~~ part of the study; six patients having significant cognitive impairment attributable to LHRHa and six patients without cognitive impairment. This grouping will ~~screening~~ will be based on ~~a detailed~~ clinical history taken from the patient and/or their partners where applicable and detailed neuropsychological assessment. ~~As part of screening,~~ Ppatients will ~~also~~ be required to undergo a screening blood test to determine their genetic predisposition to high TSPO binding. Only those patients having the genetic polymorphism for high [^{11}C]PBR28 uptake will continue to the next stage of the study. Therefore dependent on the incidence of polymorphism in the population (likely 25-50%) it is envisaged that 2-4 times the number of patients will be needed to be recruited at the screening stage. Also keeping in mind that some patients may withdraw following recruitment to the second phase of the study for several reasons such as disease progression, more patients will need to be recruited in both study groups initially to get the final sample size of 12 PET scanned patients to complete this pilot study.

TREATMENT OF PATIENTS

This is not a treatment study and the study will not interfere with the patient's on-going anticancer therapy. Patients will continue the same therapy for as long as it is deemed appropriate by their treating consultant.

Medications

This is not a treatment study. All medications will be recorded and continued throughout the study unless the responsible physician decides otherwise. Any additional treatment that the responsible physician feels is appropriate is permitted.

Radiotherapy

The treating physician might start the patient on radiotherapy at any time if there is any indication; this will not interfere with the study.

PROCEDURES

STAGE 1

Stage 1 will include clinical history and neuropsychological assessment (paper and computer-based ~~and online~~). Blood tests for determining TSPO (Ala147Thr) polymorphism

and sex hormone levels~~, full blood count (FBC) and coagulation profile~~ will also be done at~~during~~ this stage. All stage 1 procedures will be carried out at the uro-oncology clinics of ~~Charing Cross hospital~~ICHT on routine follow-up visits. Based on results from Stage 1, eligible patients will be invited to proceed to the next stage ~~of the study~~.

Neuropsychological assessment

Neuropsychological functions will be assessed by using a battery of paper-based standard neuropsychological questionnaires and ~~online~~ computerised detailed remote tracking of cognition and emotion~~ via CBSTrials.com.~~ The paper and computer-based ~~and online~~ assessments will be administered to patients~~, and their spouse or partner where available.~~

The detailed neuropsychological battery will be used to assess current verbal and nonverbal reasoning ability, cognitive flexibility, inhibition, set shifting, executive functions, working memory, verbal recall, associative learning and recall.

~~Online assessments will be completed by the patients and their spouse or partner at home. For the internet-based cognitive testing, participants and their family members will receive an email reminding them that it is time to be tested. Clicking on the email link will take them directly into the set of cognitive tests. They will then be presented with the tests one after the other in a pre-set sequence. When all tests are completed, they will be navigated to a simple end page. Additional exclusion criteria will be used for this component of the study. Participants having internet access at home will be enrolled.~~

Based on clinical history and neuropsychological assessments, patients will be grouped into two arms, (1) those having significant MCI due to ADT and (2) those without evidence of MCI.

Determination of TSPO polymorphism

For determining TSPO (Ala147Thr) polymorphism, 10ml venous blood will be collected in EDTA tubes and the sample will be sent for analysis to the Hematology department, Hammersmith hospital within 72 hours of collection. Only those patients shown to have the genetic polymorphism demonstrating expression of high affinity 18 kDa TSPO/Peripheral benzodiazepine receptor for binding the PET imaging marker[^{11}C]PBR28~~.~~ will progress to stage 2.

Hormone levels

A venous blood sample (10ml) for measurement of hormones (testosterone and oestradiol) will be obtained.

~~*Coagulation Profile and FBC*~~

~~A coagulation profile (PT, APTT) and a full blood count (FBC) will also be performed to ensure that the subject is eligible for an arterial cannulation.~~

STAGE 2

Stage 2 will involve ~~arterial cannulation and~~ research imaging of the brain to be undertaken at the Imanova Centre for Imaging Studies. Patients will be registered at the Hammersmith hospital before the imaging session and then will undergo a brain PET scan and a brain MRI scan on the same day at the same visit.

~~**Arterial Cannulation**~~

~~An arterial line will be inserted for blood sampling during the PET scan. In case of failure to insert an arterial line, we will either not go ahead with the PET scan or we may~~

~~decide to scan without arterial lines if preliminary results are available for us to get meaningful data without arterial cannulation.~~

PET Scan

During the PET scanning session, an x-ray topogram will be performed to localise the area to be scanned. This will be followed by a CT scan localised to the area to be scanned for attenuation and scatter corrections followed by administration of [11C]PBR28 and dynamic PET imaging for 90 minutes. Target dose of [11C]PBR28 will be 400MBq. ~~Continuous and discrete arterial blood samples will be taken throughout the duration of the [11C]PBR28 scans for measurement of total plasma and whole blood radioactivity and for radiolabelled metabolites. The total amount of blood withdrawn during the course of the PET scanning would be about 135 ml (75ml for continuous arterial blood sampling (15min @5 ml/min) + 57ml for discrete sampling for a 90min scan = ~132 ml).~~

PET images will be reconstructed using the filtered back projection (FBP) and ordered subset expectation maximisation (OSEM) algorithms. ~~Regions of interest (ROIs) will be defined outlining the area of interest and will be applied to the dynamic [^{11}C]PBR28 data to generate mean voxel radioactivity for the full duration of the scan. Time activity curves for the full duration of the scan corrected for radioactive decay and normalized for injected radioactivity will be generated.~~ Tissue uptake parameters including but not limited to SUV ~~and VT~~ will be calculated. ~~[11C]PBR28 input function derived from the time course of arterial blood radioactivity will be used to model and obtain VT.~~

In the case of [^{11}C]PBR28 preparation failure, the PET scan will be done later on the same day if feasible, or on another day. Patients will be informed beforehand that there is up to a 5% scan failure rate.

MRI Scan

A structural MRI scan will be performed for neuroanatomical interpretation of the PET scan. No contrast material will be used. Checks will again be made before scanning to ensure that no patient has any MRI-incompatible metallic object in his body. Additionally, rest and task-activated fMRI measures will be performed and the total scan time will be one hour approximately.

STATISTICS AND DATA ANALYSIS

This is a pilot study with a sample size for PET and MRI studies limited to 12 patients. The ~~kinetically~~ acquired PET data~~, requiring accompanying arterial blood sampling, will be processed to provide parametric images of the ligand's binding potential or equilibrium distribution volume. Statistical parametric mapping (SPM) of the quantitative images of binding potential or equilibrium distribution volumes of [^{11}C]PBR28~~ will be used to identify ~~regional effects~~differences in ligand binding between the two patient arms and of the combined data from the two groups against fMRI measures and cognitive scores. This will inform the design of a future more comprehensive and definitive study and likely identify underlying mechanisms which could then be further investigated. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study.

END OF STUDY

The study will be considered 'closed' at the last visit of the last subject (LVLS).

WITHDRAWAL OF PATIENTS

Every patient has the right to withdraw consent for participation in the study at any time. A patient may withdraw, or be withdrawn, from trial participation for the following reasons:

1. Intercurrent illness which prevents participation.
2. Withdrawal of consent.
3. Any alteration in the patient's condition which justifies the discontinuation in the clinician's opinion.

CLINICAL QUERIES

Clinical queries should be directed to the Chief Investigator who will direct the query to the appropriate person.

INICIDENTAL CLINICAL FINDINGS

The scans in this study are not aimed at diagnosing medical disorders. However, there is a small probability of incidental findings in the structural MRI scans and the attenuation CT scans of the PET-CT scan performed as part of the study. Therefore all scans will be reviewed soon after acquisition by an Imperial College Healthcare NHS Trust consultant radiologist and incidental findings reported to the CI. The CI will arrange for any such patient to be seen in clinic for counselling about the incidental finding(s) and, if consent is given, inform the patient's GP for further action.

ADVERSE EVENTS 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** – *refers to an event in which the subject 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*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

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

REPORTING PROCEDURES FOR ADVERSE EVENTS

All adverse events should be reported. 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. Any adverse events arising as a result of the radiotracer administration will be reported to Imanova as per the Imanova standard operating procedures.

Non serious AEs

All such events, whether expected or not, should be recorded.

Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to prostate cancer and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.
All SAEs should be reported to the 'name of 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.

Contact details for reporting SAEs

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REGULATORY ISSUES

Ethics Approval

The Chief Investigator will obtain approval from the London Queen Square Research Ethics Committee. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. 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. Except in the case of urgent safety measures, any amendments to the protocol should be approved by the sponsor, prior to submission to the ethics committee. Upon receiving ethical approval, any amendment must receive further Trust approval, before being implemented at site. In the case of an urgent safety measure, the sponsor and REC should be informed as soon as possible following the amendment.

ARSAC Certification

Approval from the Administration of Radioactive Substances Advisory Committee (ARSAC) at the National Radiation Protection Board to administer radioactivity has been obtained for the study. Radiotracer prescription will be signed by the ARSAC holder or an authorized delegated investigator.

Consent

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent must be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time without giving reasons and without prejudicing further treatment.

Confidentiality

All information collected during the course of the research will be kept strictly confidential. The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. In addition, all procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act. No individual patients will be identified when the results of the trial are published.

Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study. Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study.

Sponsor

Imperial College London is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 5L10C, 5th Floor Lab Block
Charing Cross Hospital, Fulham Palace Road
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Funding

The funding for the research will be provided by a joint Imanova / BRC grant. Dr SIA Shah is funded by an IC commonwealth scholarship.

Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by the Chief Investigator.

PUBLICATION POLICY

The results from the study will be analysed and published in a journal article. The results will also form part of a doctoral thesis. All publications will acknowledge the participating institutions and clinicians.

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