BIOPIC: Biological Magnetic Resonance Imaging Parameters in oropharyngeal squamous cell carcinoma

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Declaration of interests: Nil to declare

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY CONTACTS

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2. SYNOPSIS

Study Title	Biological Magnetic Resonance Imaging Parameters in oropharyngeal squamous cell carcinoma	
Short Title	BIOPIC	
Rationale:	Imaging is a vital component of the head and neck cancer management pathway. Magnetic Resonance Imaging (MRI) is used routinely to provide anatomical assessment and with the emergence of novel techniques may be used for imaging physiological processes. This study will focus on MRI with supplemental oxygen/carbogen breathing and assessment of exogenous and endogenous contrast agents with Chemical Exchange Saturation Transfer (CEST) MRI in patients undergoing radical dose radiotherapy. Multiparametric tissue characterisation with MRI has potential for use in adaptive radiotherapy planning, to assess the effects of treatment and evaluate the actions of targeted therapies.	
Study Design	Observational	
Study Participants	Patients with HPV positive oropharyngeal squamous cell carcinoma undergoing radical radiotherapy.	
Sample Size	20	
Follow-up	3 months	
	Objectives	Outcome Measures
Primary	Assess changes in tumour T1 & T2* imaging with supplemental oxygen during and after radiotherapy	Changes from baseline in T1 & T2* signal during and post RT with supplemental oxygen
Secondary	Assess changes in CEST imaging with and without supplemental oxygen during and after radiotherapy	Changes from baseline in CEST signal during and post RT with and without supplemental oxygen
	Assess changes in tumour T1/T2* & CEST imaging with supplemental carbogen during and after radiotherapy	Changes from baseline in T1, T2* and CEST signal during and post RT with supplemental carbogen
	Assess changes in CEST imaging with treatment	Changes from baseline during week 2 RT and 10 weeks post RT - GlucoCEST levels pre & post glucose load - Markers such as Choline, creatine, lactate, APT

	Assess whether glucoCEST imaging correlates with FDG-PET parameters	Compare glucoCEST levels with FDG-PET parameters including SUVmax & uptake volume at baseline & 10 weeks post RT
	Compare dynamic contrast enhanced MRI with dynamic glucoCEST	Standard DCE-MRI parameters (e.g. ktrans, Kep, Ve, AUC) will be compared with dynamic glucoCEST signal changes (e.g. AUC).
	Evaluate the consistency of MRI parameter measurements	Test-retest imaging at baseline in subgroup of participants
Exploratory	Compare imaging findings with histopathology	Immunohistochemistry and genetic markers including CAIX, Ki-67 and GLUT-1. Other markers such as those related to hypoxia, proliferation, radioresistance and metabolism will be explored.
	Evaluate the use of circulating biomarkers in head and neck cancer	Investigational work to identify potential circulating biomarkers to include circulating tumour DNA/RNA. Compare to tissue genetic markers.

3. ABBREVIATIONS

AE	Adverse event
APT	Amide Proton Transfer
AR	Adverse reaction
CEST	Chemical Exchange Saturation Transfer
CI	Chief Investigator
CRF	Case Report Form
CRT	Chemoradiotherapy
CTRG	Clinical Trials and Research Governance
DCE	Dynamic Contrast Enhanced
FDG-PET	^{18}F-Fluorodeoxyglucose Positron Emission Tomography
GCP	Good Clinical Practice
GP	General Practitioner
Gy	Gray
HNSCC	Head and neck squamous cell carcinoma
HPV	Human Papilloma Virus

ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMRT	Intensity Modulated Radiotherapy
IRB	Independent Review Board
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NRES	National Research Ethics Service
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RT	Radiotherapy
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
SUV	Standardised Uptake Volume
T1	MRI sequence based upon the recovery of longitudinal magnetisation of protons (small particles that form part of the nucleus or centre of an atom)
T2*	MRI sequence based upon the decay of transverse magnetisation of protons, which may be affected by magnetic field inhomogeneities

4. BACKGROUND AND RATIONALE

Head & neck cancer is the seventh most common cancer type in the UK, leading to >3,300 deaths/year. 90% are squamous cell carcinomas with oropharyngeal being the most commonly affected subsite (1). The incidence of oropharyngeal cancer more than doubled from 1990 to 2006 and changes in the pattern of causation have been demonstrated, with the role of human papilloma virus (HPV) now recognised. Although survival rates have been improving, 5 year relative survival rates are 52% and patients undergoing treatment with radiotherapy and surgery may suffer significant morbidity as a result of treatment (2). This study will assess the role of novel MRI techniques in patients undergoing radical dose radiotherapy (with or without systemic therapy) for HPV positive oropharyngeal cancer.

Imaging is a vital component of the head and neck cancer management pathway, used for diagnosis, staging, treatment planning and response assessment. Magnetic Resonance Imaging (MRI) is commonly used due to the anatomical detail provided. In addition, functional imaging such as FDG-PET is being increasingly utilised for detecting the primary site in patients presenting with occult disease, regional nodal, and distant metastases. Both modalities are used routinely to assist CT-based radiotherapy

planning in head and neck cancer. MRI is superior to other imaging modalities in its ability to perform multiparametric tissue characterisation and in addition does not involve ionising radiation. Functional MRI, combining physiological information with anatomical detail, is therefore ideally placed for use in adaptive radiotherapy planning, with further potential to assess the effects of treatment and evaluate the actions of targeted therapies. MRI technology has advanced considerably over recent years with improvements in speed and quality of data acquisition, image reconstruction and post processing. MRI sensitivity and specificity are dependent not only on technique and verification standards but also patient selection and field strength. When compared to 1.5T, 3T has the added benefit of a higher signal to noise ratio permitting higher resolution imaging and can generally detect lesions as small as 3mm (3). Although there are some technical drawbacks with higher magnet strength MRI scanners, their increasing availability provides an opportunity to develop relevant sequences and establish clinical benefit. There is significant potential for quantitative MRI assessment of features important in cancer pathophysiology. With the advent of modern machines and techniques, their practical use in the clinical setting is promising.

In this study, two novel MRI techniques which assess features affecting sensitivity to chemotherapy and radiotherapy will be evaluated. T1 and T2* mapping MRI sequences with and without supplemental oxygen and carbogen (up to 5% carbon dioxide, ≥95% oxygen) will provide information about tumour oxygenation. Chemical Exchange Saturation Transfer (CEST) MRI allows assessment of concentrations of molecules such as glucose and amide proton transfer (APT), which reflect metabolism and pH respectively. The ability to highlight areas less likely to respond to radiotherapy provides an opportunity to target them using Intensity Modulated Radiotherapy (IMRT) or adaptive RT, or to use appropriate targeted radiosensitisers.

Hypoxia (deficiency in amount of oxygen reaching tissue) is a common feature of solid tumours and is linked to chemo- and radio-resistance along with a more aggressive phenotype. Limited perfusion due to inadequate vasculature is a contributory factor (4). The ability to characterise these features provides the potential to target and modulate these aspects of the local environment in order to improve cancer control. Oxygen is a weakly paramagnetic agent which may be used as a contrast agent for MRI. Supplemental oxygen breathing may provide additional information about tumour oxygenation and does not require the use of exogenous gadolinium contrast agents (5, 6). Hyperoxia initially directly affects the T1 relaxation of arterial blood, and over longer periods can affect the T1 in tissues as local PO_2 increases (7). In malignancy, O'Connor et al. found generally good agreement between the spatial distribution of oxygen-enhanced and dynamic contrast enhanced (DCE) MRI (6). There were some areas of mismatch, suggesting that significant hyperoxic T1 enhancement may reflect perfusion, whereas a lack of enhancement may indicate regional tumour hypoxia. Hyperoxia also affects T2* MRI signal due to changes in the oxy/deoxyhaemoglobin ratio (8). Jiang et al. used this technique to assess response to chemotherapy in breast cancer and found that hyperoxic T2* provided additional information regarding tumour oxygen status when compared to DCE-MRI measures of perfusion (5)**.** Further information may be provided by the addition of 5% carbon dioxide to oxygen (carbogen), which causes vasodilatation and a shift of the haemoglobin-oxygen dissociation curve to the right (9). This leads to more oxygen being available in the capillaries and a subsequent increase in image signal intensity. Correlation between the presence of tumour and T2* signal enhancement with carbogen breathing has been demonstrated in

brain (10) and prostate cancers (11). The latter postulated heterogeneity of response to carbogen as indicating variation in tumour vascularisation. Thus a combination of T1 and T2* imaging with supplemental oxygen and carbogen may provide complementary information reflecting tissue and vascular oxygenation.

Chemical exchange saturation transfer (CEST) MRI is a novel sequence which provides a means of inducing image contrast proportional to the local concentration of compounds through the saturation of off-resonant exchanging protons (12). This can be the result of either endogenous or exogenous agents, and provides a mechanism by which specific biomarkers of disease can be imaged at concentrations as low as 1 mM at standard MRI resolutions of 1 mm^3 (13). CEST images have been successfully acquired in patients with head and neck cancer by Wang et al (14). In the Churchill Hospital, Oxford, we have undertaken CEST imaging of the normal brain in volunteers and demonstrated largely uniform CEST signal changes in grey matter compared to white matter with supplemental oxygen breathing. We intend to assess the potential of OxyCEST and explore the use of carbogen with CEST to demonstrate localised changes in tumours compared to normal tissue.

As CEST MRI provides the ability to measure multiple compounds in a relatively rapid, non-invasive, non-ionising fashion, it may allow for identification and monitoring of multiple potential biomarkers. CEST imaging using glucose as a contrast agent (glucoCEST) was found by Walker-Samuel et al. (15) to be comparable to FDG-PET autoradiography in human colorectal tumour xenograft mouse models. In patients with brain tumours, Xu et al (16) demonstrated varying enhancement both spatially and with time using dynamic glucoCEST imaging. They suggest that dynamic glucose enhanced (DGE) MRI may provide information regarding tumour glucose uptake but it is sensitive to perfusion-related properties, which can be assessed by DCE-MRI. Contemporaneous DGE and DCE will therefore be undertaken in the BIOPIC study.

FDG-PET is used routinely in the assessment of head and neck cancer patients but it is time-consuming and involves ionising radiation; MRI may represent a potential alternative modality with the ability to measure not only glucose but other metabolic markers such as lactate, creatine and choline. The phosphocreatine/creatine kinase system plays a key role in cellular energy buffering and transport, especially in cells with high and disturbed energy metabolism. MR spectroscopy of head and neck squamous tumour specimens found that the choline to creatine ratio was significantly elevated in the group with poor response (17). In addition, Yuan et al. (2014) (18) have demonstrated the feasibility and potential clinical application of APT CEST in head and neck tumours. Amide proton transfer (APT) CEST allows for the determination of relative pH non-invasively (19) and has been successfully used to detect and grade tumours (20, 21), distinguish oedema from brain tumour (22) and characterise ischaemia (23). This study will evaluate the potential of CEST MRI to characterise HNSCC and assess response to treatment.

Next generation sequencing techniques now available have the ability to measure circulating tumour genetic material. The potential to use cfDNA to predict response to therapy offers a non-invasive tool of assessment. Furthermore, it may allow more detailed information on the change in tumour biology with

therapy and tumour heterogeneity. We will investigate the feasibility of performing these analyses to assess changes in tumour genetics and driver mutations in patients undergoing chemoradiotherapy. In head and neck cancer, a variety of circulating markers have been assessed including miRNA (24), circulating tumour cells (25) and DNA (26). For analysis of circulating tumour DNA and RNA in this study, research blood samples will be taken at each imaging visit and compared to immunohistochemical and genetic analysis of routine tissue samples where available.

5. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures**	**Time point(s) of evaluation**
Primary Objective		
Assess changes in tumour T1 & T2* imaging with supplemental oxygen during and after radiotherapy	Changes from baseline in T1 & T2* signal during and post RT with supplemental oxygen	Imaging at baseline, week 2 RT and 10 weeks post RT
Secondary Objectives		
Assess changes in CEST imaging with and without supplemental oxygen during and after radiotherapy	Changes from baseline in CEST signal during and post RT with and without supplemental oxygen	Imaging at baseline, week 2 RT and 10 weeks post RT
Assess changes in tumour T1/T2* & CEST imaging with supplemental carbogen during and after radiotherapy	Changes from baseline in T1, T2* and CEST signal during and post RT with supplemental carbogen	Imaging at baseline, week 2 RT and 10 weeks post RT
Assess changes in CEST imaging with treatment	Changes from baseline during and post RT in: • GlucoCEST pre & post glucose load • Markers such as Choline, creatine, lactate, APT	Imaging at baseline, week 2 RT and 10 weeks post RT
Assess whether glucoCEST imaging correlates with FDG-PET parameters	Compare glucoCEST signal changes with contemporaneous FDG-PET parameters including SUVmax, uptake volume	Imaging at baseline and 10 weeks post RT

Compare dynamic contrast enhanced MRI measures of perfusion with dynamic glucoCEST	Standard DCE-MRI parameters such as ktrans, Ve, AUC will be compared with dynamic glucoCEST signal changes such as AUC.	Imaging at baseline, week 2 RT and 10 weeks post RT
Evaluate the consistency of MRI parameter measurements	Test-retest imaging at baseline in a subgroup of participants	Repeat imaging at baseline
Exploratory Objectives		
Compare imaging findings with histopathology	Immunohistochemistry and genetic markers including CAIX, Ki-67 and GLUT-1. Other markers such as those related to hypoxia, proliferation, radioresistance and metabolism will be explored.	Pre-treatment biopsy Post-treatment biopsy or surgical specimen (where applicable)
Evaluate the use of circulating biomarkers in head and neck cancer	Investigational work to identify potential circulating biomarkers including circulating tumour DNA/RNA. Compare to tissue genetic markers.	Blood sampling at each imaging time point

6. STUDY DESIGN

This is an observational study to assess novel MRI imaging techniques T1 and T2* with supplemental oxygen and carbogen breathing and CEST. Baseline scans will be compared to imaging during and post treatment in patients undergoing radiotherapy for oropharyngeal squamous cell carcinoma. For glucoCEST, comparison to DCE-MRI and contemporaneous clinical FGD-PET will also be undertaken. An exploratory study will investigate immunohistochemical and genetic markers related to factors influencing response to radiotherapy and compare these to findings on imaging.

Patients will be recruited from a single centre (Oxford University Hospitals Foundation Trust) and will receive standard treatment and follow-up as part of routine NHS care. 15 participants (group A) will undergo study MRI lasting no more than one hour at three time points (baseline, during and after radiotherapy). A subgroup of participants (group B) will have an additional MRI at baseline i.e. 4 study MRI scans in total. Every patient who agrees to participate in the study and is felt by the investigator to be suitable will be asked if they are willing to be part of group B until a total of 5 participants is reached. From seeking informed consent, patients will be followed until post-treatment imaging which takes place 10-12 weeks after completion of radiotherapy. The duration of patient participation will be approximately 4 months. See Appendix A for study flowchart.

7. PARTICIPANT IDENTIFICATION

7.1. Study Participants

Participants with HPV positive oropharyngeal squamous cell carcinoma who are to receive radical dose radiotherapy +/- systemic therapy will be recruited.

7.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 18 years or above.
- HPV positive oropharyngeal squamous cell carcinoma for treatment with radical radiotherapy +/- systemic therapy.
- The tumour is at least T2 or if nodal disease is used as the treatment assessment site, the node is at least 2 cm in minimum diameter on MRI.
- In the Investigator's opinion, is able and willing to comply with all study requirements.
- Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study.
- Involvement in other clinical studies is acceptable

7.3. Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Female participant who is pregnant, lactating or planning pregnancy during the course of the study.
- Significant renal or hepatic impairment.
- Type 1 diabetes mellitus or poorly controlled T2 diabetes mellitus or fasting capillary/venous blood glucose level >8mmol/L.
- Ongoing supplemental oxygen as part of clinical care
- Known lung disease with carbon dioxide retention, chronic obstructive airways disease with known or at risk of hypercapnia
- Most recent available arterial blood gas (ABG) from the current hospital admission demonstrates hypoxia or hypercapnia on room air.
- Any patient not felt to be suitable for supplemental oxygen or carbogen as considered by an appropriately trained clinician.

- Contraindication to MRI (e.g. cardiac pacemaker, ferromagnetic cerebral aneurysm clip, metallic foreign body in the eye)
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.

8. STUDY PROCEDURES

8.1. Recruitment

Following confirmation of diagnosis and management plan (usually by the Multidisciplinary team), potential participants will be approached during routine clinical appointments by a member of their clinical care team.

8.2. Screening and Eligibility Assessment

All patients within the OUH NHS Trust with a clinical suspicion of HNSCC are put on the cancer pathway and undergo CT and MRI staging in addition to tumour biopsies. Once these assessments are complete the patients are discussed at the head and neck MDT team meeting and their management plan confirmed. Potential study participants will be identified on the basis of the routine evaluations performed and can be approached and given a patient information sheet from the point of being informed of their diagnosis and treatment plan onwards at the discretion of the clinical team.

8.3. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. A copy of the signed Informed Consent will be given to the participant and placed in the medical notes. The original signed form will be retained at the study site.

8.4. Baseline assessment

The following will be recorded on the CRF: demographics, medical history, recent (within last 8 weeks) clinical laboratory tests including full blood count, urea and electrolytes, liver function tests (referred to as routine bloods hereafter).

Imaging is described in section 8.5.

8.5. Study Visits & Assessments

Where possible, study visits will coincide with visits that would form part of routine care within the NHS for a patient receiving RT for HNSCC. The patient will receive all radiotherapy appointments, including planning CT appointments as per normal NHS practice.

The following visits will be required for all participants unless otherwise specified. They will be in addition to standard imaging but coincide with routine appointments where possible.

Visit 1: Consent

At least 24 hours following receipt of Patient Information Sheet. May coincide with visit 2. Medical history and current medication should be confirmed at this visit.

Visit 2: Baseline MRI & blood test

Within 21 days before initiation of radiotherapy.

Participants will be asked to fast for at least 6 hours prior to the MRI excepting sips of water. An 18 or 20 Gauge cannula will be placed in the antecubital fossa. Another cannula (20, 22 or 24 Gauge) will be placed in the contralateral hand or arm where possible. Blood samples of 40ml in total for circulating tumour markers will be taken. A member of the research team will record on the CRF if any changes to the medical history or medication have occurred and any routine blood results taken since the previous visit. The above should take approximately 10 minutes.

MRI (up to 1 hour)
The following sequences will be undertaken as indicated (any sequence may be reordered or omitted at the discretion of the investigators):

1. Localising and anatomical sequences
2. Diffusion weighted imaging
3. T1/T2* on air
4. CEST on air
5. Spectroscopy (for other metabolites)
6. T1/T2* with oxygen
7. CEST with oxygen
8. T1/T2* with carbogen
9. CEST with carbogen
10. CEST with intravenous glucose
11. Dynamic contrast enhanced MRI with gadolinium-based contrast

Each period of supplemental oxygen or carbogen breathing will last no longer than 15 minutes. Participants will wear an MRI compatible gas delivery device where required during scanning in order to supply the supplemental oxygen or carbogen gas. Patients will be monitored throughout these periods using a gas analyser and will be able to communicate with researchers in the control room.

Intravenous dextrose will be given for no more than 20 minutes. Participants will have an intravenous bolus injection of 50ml 50% (0.5g/ml) dextrose solution by a 20ml saline flush. They will then have up to a 20 minute infusion of 10%-20% dextrose solution followed by a further 20ml saline flush. The infusion may be reduced or omitted if early analysis suggests it is not required. Participants will be asked to inform research staff immediately if the cannula becomes uncomfortable or painful. Blood glucose testing using a hand-held blood glucose monitoring device will be done either by sampling through the cannula in the contralateral arm or hand to that used for contrast injection or through finger prick testing. Blood sampling will be undertaken once prior to MRI (twice if the first result was out of the expected range) and up to four times during the MRI on each scan visit.

Visit 3: Baseline MRI 2 (Group B only)

Group B participants will have a second baseline scan at least 48 hours following the first, for test-retest purposes. A blood test will be taken at this visit if not done at visit 2.

Visit 4: On treatment MRI & blood test

On fraction 10+/-2 of radiotherapy. Prior to radiotherapy that day where possible. The MRI and blood test will be undertaken as on Visit 2 (baseline).

Visit 5: Post-treatment MRI & blood test

To coincide with routine post-treatment imaging where possible. The MRI and blood test will be undertaken as on Visit 2 (baseline).

The patient's participation in the study will end following their post-treatment MRI or at time of radical surgery if they proceed to resection (expected only in a minority of cases).

8.6. Information gathering from medical notes, online systems and databases

Information including cancer stage, treatment given (radiotherapy, chemotherapy, biological agent) and response to treatment will be recorded on the CRF as will results of investigations including pathology, blood tests and imaging.

8.7. Handling of imaging data

Once a scan has been performed, images will be anonymised by replacement of all clinical identifiers with the clinical trial number. The anonymised images will be transferred for long term storage in a server within the

University of Oxford. In collaboration with imaging scientists, detailed evaluation of the images will be undertaken to characterise tumour changes with radiotherapy. These evaluations of the tumour will not directly influence the management of the patient in any way. Should new information emerge from the imaging investigations employed in this study that would impact upon the management of the patient (e.g. detection of previously unknown metastatic disease), the patient would be withdrawn from the study and the appropriate clinical team alerted. Radiological measurements of the extent of disease by MRI, CT of the chest, abdomen and pelvis and whole body FDG-PET will also be performed as per routine clinical practice. These routine diagnostic scans and reports as well as radiotherapy planning imaging will be anonymised and stored on the university server as part of the trial record.

8.8. Sample Handling

Blood samples will be collected, labelled and transferred as per the procedure of the Oxford Radcliffe Biobank. The date and time of collection will be recorded on the CRF. Samples will be stored analysed within the timeframe after collection for which the stability in the samples has been validated and found acceptable.

Consent will be requested from all participants for access to NHS biopsies and surgical specimens that have been or will be obtained as part of routine clinical care, either prospectively or retrospectively. Resection specimens may be transferred to an HTA licensed biobank while the study is open to recruitment and up to 24 months from closure of the study to recruitment for possible use in future ethically approved research. Immunohistochemical assessment of the biopsies and resection specimens will be performed. Genetic analyses will be performed in tissue obtained from consenting individuals to study somatic mutation profiles and gene expression profiles of response, with suppression of genes related to heredity. Data relating to genes known to carry heritable information will be discarded and deleted permanently from the raw data. This work is aimed at the discovery of new tissue biomarkers that may complement the novel imaging modalities being developed in this project.

	ASSAY 1	ASSAY 2	ASSAY 3
Purpose of assay	Genomics	Fixation/storage of biopsy/ resection tissue	Fixation/storage of biopsy/resection tissue
Assay	Genetic sequencing	Immunohistochemistry	Genetic sequencing
Type of sample	Whole blood	Biopsy/resection tissue	Biopsy/resection tissue
Vials used	10ml EDTA	Standard	Standard
Total volume/visit	40ml	Standard	Standard
Total volume/patient	120ml	Standard	Standard
Timepoints	Pre, during, post treatment	As per clinical standard	As per clinical standard
Clinical Handling/Storage/ Transport	To sample handling/ GCP lab at room temperature	As per clinical standard or Biobank if transferred	As per clinical standard or Biobank if transferred
Storage Conditions	-70^0 freezer	As per clinical standard	As per clinical standard
Person Responsible for analysis	Chief Investigator	Chief Investigator	Chief Investigator
Frequency of analysis	Batch analysis every 5-10 patients	Batch analysis every 5-10 patients	Batch analysis every 5-10 patients

8.9. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements
- An adverse event which results in inability to continue to comply with study procedures
- Disease progression which results in inability to continue to comply with study procedures
- Withdrawal of Consent
- Loss to follow up

The treating consultant will be made aware of the participant's withdrawal from the study. The data from that participant may be included for study endpoints if appropriate and at the discretion of the investigator. The participant can be replaced if they do not complete all three MRI scans mandated in this study. Participants may continue on the study without carbogen breathing if this is not tolerated. They may be replaced at the discretion of the Investigator.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

8.10. Definition of End of Study

The end of study is the date of the last visit of the last participant.

9. SAFETY REPORTING

9.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.

9.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

9.3. Management of expected adverse events

We do not anticipate any adverse events from the investigational scans. MRI does not require the use of ionising radiation and standard MRI precautions and exclusions will be followed to ensure the safety of study participants.

In terms of supplemental oxygen and carbogen, once contraindications are excluded, the risks of taking part in such experiments are minimal. A researcher will go through a list of possible risks with the participant before the study and answer any questions. Oxygen or carbogen will be delivered through either a breathing mask or a nasal cannula, termed the gas delivery device. The breathing mask is placed over the nose and mouth and secured with a strap behind the head. The nasal cannula consists of a pair of gas outlets that are placed just below the nostrils which are secured by connected tubing that runs over and behind the ears. In both cases, gases are transported via tubing to the gas delivery device. Delivery of gases will be controlled by a system of manual or automated control valves. Recent Cochrane reviews highlight the lack of evidence on either beneficial or detrimental effects of oxygen (27, 28). Within the scope of these findings, breathing of normobaric supplementary oxygen of up to 100% for short periods is considered completely safe both in normal controls and patient groups. The risk of oxygen toxicity would only become of importance beyond 16 hours of oxygen administration and at partial pressures beyond 0.5 bar. MRI scanning of patients breathing supplemental oxygen is clinically routine and standard precautions will be taken. The range of hypercapnia stimuli that will be used in this study is physiological and is experienced repeatedly by most people throughout the day in the course of their lives. Supplemental carbogen breathing can induce the sensation of breathlessness and patients will be warned of this potential effect. During the whole duration of the experiment, the participant is able to talk with the researchers and indicate any discomfort. They will be monitored continuously during supplemental oxygen and carbogen breathing using a gas monitor.

In order to provide contrast for the glucoCEST MRI imaging, participants will be asked to fast for a period of at least six hours prior to intravenous dextrose being administered: 50ml of 50% dextrose bolus followed by 100ml of 10%-20% dextrose over 10-20 minutes, total 45g glucose. If after early analysis the infusion is not required, it will be omitted for future patients. The bolus being given is analogous to that used for the intravenous glucose tolerance test (IVGTT) and the total dose of 45g glucose is within the

range used as part of the IVGTT. The IVGTT has been extensively studied for use in the diagnosis of diabetes mellitus (29, 30) and is not expected to cause significant risk to participants. There have been reports of thrombophlebitis following the peripheral administration of intravenous dextrose and to limit this, cannulation of small veins will be avoided and an 18 or 20 Gauge cannula should be used. All patients will receive a 20ml saline flush immediately following both the dextrose bolus and infusion. Patients with diabetes mellitus type 1 or poorly controlled type 2 diabetes mellitus will be excluded from taking part as will any participant with a confirmed fasting blood sugar of 8mmol/L. Blood sugar levels will be checked before and during the MRI scan as detailed in section 8. If 2 abnormal glucose results are obtained at baseline or the investigator has concerns about proceeding, the patient will not receive the intravenous dextrose. The participant may continue with the remaining MRI sequences & visits at the discretion of the investigator. The administration of intravenous dextrose and blood glucose testing will be done for study purposes only and will not determine patients with previously undiagnosed impaired glucose tolerance or diabetes mellitus. Any concerns will be reported to the patient's GP who may then arrange further investigations.

Gadolinium is in routine clinical use as an intravenous MRI contrast agent. Standard hospital policy will be followed when used in this study. It will not be given to patients with significantly reduced renal function. It can cause side effects but these are usually mild and short-lasting. These include injection site pain, nausea, vomiting, itching, rash, headache and paraesthesia (abnormal skin sensation). If the injection leaks out from the vein into surrounding tissue (extravasation), patients may experience stinging or pain. Allergic reactions are uncommon but can occur. Extravasation and allergic reaction will be managed as per standard hospital policy if they occur.

Participants will be assured that they can withdraw from the study for any reason without penalty.

10. STATISTICS

10.1. Description of Statistical Methods

Descriptive statistics of the patient population will be reported using numbers (with percentages) for binary and categorical variables and means (and standard deviations), or medians (with lower and upper quartiles) for continuous variables. Measurements from the different MRI sequences, FDG-PET scans, immunohistochemistry and genetic expression analyses will be described and the association between them will be assessed using appropriate measures of correlation.

The primary outcome measure is to assess percentage change from baseline in T1 and T2* during and post RT, with supplemental oxygen. Secondary outcomes measures include the percentage change in CEST signal with the addition of supplemental oxygen and carbogen, the percentage change in T1 and T2* with supplemental carbogen and the percentage change in glucoCEST signal with intravenous glucose. Changes in GlucoCEST signal with treatment will be compared to changes in DCE-MRI parameters, including ktrans, and FDG-PET parameters, including SUVmax. In Group B, we will be testing intra-patient agreement between sequential scans. The Bland-Altman test will be used to quantify the degree of agreement between the imaging parameters derived from the different sequences. There are no planned interim statistical analyses.

10.2. The Number of Participants

20 patients will be recruited in total. 15 patients will be recruited into Group A and 5 patients will be recruited into group B and undergo two baseline scans. This sample number has been chosen to give reasonable assessment of the endpoints in this observational study exploring novel MRI techniques in radiotherapy within a reasonable time frame. The results from this study will be used to plan future studies.

Based upon projected accrual rates, this study is expected to complete recruitment within 18 months of opening to recruitment. Patients who do not complete the study may be replaced if within the planned recruitment period at the discretion of the Chief Investigator.

10.3. Analysis of Outcome Measures

Primary and secondary outcomes will be analysed as described in section 10.1. Data from group A patients will be analysed for all endpoints except the secondary endpoint related to test-retest imaging. Data from group B participants will be used to address all endpoints. All participants will be included in the analysis of data, whether or not they undergo all of the planned imaging studies.

11. DATA MANAGEMENT

11.1. Source Data

Source documents include hospital records and correspondence, laboratory and pharmacy records, clinical imaging and reports. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). Source data will be accessed through online and paper hospital records and the CRF. All documents will be stored safely in confidential conditions. The imaging data will be archived as per department policy on the hospital Trust PACS/HERMES system.

11.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

11.3. Data Recording and Record Keeping

All study data will be entered on to paper CRFs and/or the study database which will be stored on a secure server with restricted access.

The participants will be identified by a unique study specific number and/or code in any database. This will be assigned at the time of entry into the trial, following the consent form being signed and prior to the first study specific procedure. The log of trial IDs will be kept in a locked office within the Oxford Imaging Trials Unit, Department of Radiology, Churchill Hospital, Oxford. It will be accessible to investigators and research unit staff. Hard copies of CRFs will be kept separately in a secure office within

the Oxford Imaging Trials Unit and the data subsequently entered onto the study database. Electronic data will be fully anonymised and kept on a password protected study database stored on trust servers. This will be uploaded to a secure University server for analysis. The imaging data will be archived as per department policy on the hospital Trust PACS/HERMES system. Imaging data will be anonymised prior to being transferred to a password protected databased on a secure University of Oxford server. The participants will be identified by a study specific participant number in any database.

Personal data recorded on all documents will be regarded as confidential, and to preserve each patient's anonymity, only their initials and date of birth will be recorded on the CRFs. The Investigator site must maintain the patient's anonymity in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled patients' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally.

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable the conduct of a clinical trial and the quality of the research data to be evaluated and verified. All essential documents must be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed. The medical files of trial subjects shall be retained in accordance with national legislation and in accordance with the host institution policy. Retention and storage of laboratory records for clinical trial samples must also follow these guidelines. Retention and storage of central laboratory records and the disposition of samples donated via the trial must also comply with applicable legislation and Sponsor requirements.

It is the University of Oxford's policy to store data for a minimum of 5 years. Investigators may not archive or destroy study essential documents or samples without written instruction from the Trial Office.

12. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

Monitoring will involve data evaluation for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

13.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

13.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities and host institution for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

13.4. Reporting

The CI shall submit once a year throughout the clinical study, or on request, an Annual Progress Report to the REC, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the REC, host organisation and Sponsor.

13.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

13.6. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

13.7. Other Ethical Considerations

Potential participants for the imaging study will be approached by the healthcare team during a routine clinical visit. They will normally be approached by a member of the Oncology team when attending for discussion regarding radiotherapy for their cancer. At this appointment, they are usually already aware of their diagnosis and the suggested management plan. Provided the member(s) of the healthcare team seeing the patient feel it appropriate, they will ask the patient for permission for a researcher to discuss the study and provide an information sheet. A researcher will then explain the study, answer any questions and provide contact information. Consent will be obtained by an appropriately trained member of the research team at least 24 hours after provision of the patient information sheet.

The on-treatment scan is done within week 2 or early in week 3 of radiotherapy. At this stage of therapy, patients are not expected to have significant side-effects such that undertaking additional scans would be

particularly uncomfortable or difficult. The final study scan will be undertaken at around 12 weeks post-treatment (where possible on the same day as routine clinical imaging), at which point the acute effects of radiotherapy would be expected to have largely or completely settled.

Participants in this trial are not expected to benefit directly in this study although future patients may do so. Participation will require extra scans and/or time spent in the scanner. To minimise inconvenience, study scans will be scheduled to coincide with routine hospital visits or scans wherever possible. Blood samples will be taken where possible from the cannula inserted for contrast injection. Any findings which may have an impact on patient management will be communicated to the relevant clinical team.

14. FINANCE AND INSURANCE

14.1. Funding

This study is part of the translational cancer research programme of the University of Oxford and the Oxford University Hospitals NHS Trust. It is undertaken via the core clinical and research infrastructure underpinned by strategic research programme grant funds. The research imaging is enabled by funding provided by the Oxford Cancer Imaging Centre (OCIC). OCIC is funded by a strategic initiative from Cancer Research UK jointly with the Engineering & Physical Sciences Research Council, the Medical Research Council and the Department of Health. Funding has also been provided by the Oxfordshire Health Services Research Committee (OHSRC), a subcommittee of the Oxford Radcliffe Charitable Funds Charity. We acknowledge support provided by The CRUK Oxford Cancer Research Centre and The Oxford National Institute for Health Research Biomedical Research Centre.

14.2. Insurance

The University of Oxford has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

15. PUBLICATION POLICY

The Trial Management Group will retain ownership of all data arising from the study. The intention is to publish this research in a specialist peer reviewed scientific journal on completion of the study. The results may also be presented at scientific meetings and/or used for a thesis. No study results may be published or presented without the prior approval of the TMG. The assigned Authors will ensure that the CI and other designated reviewers approve all study related publications. Authors will ensure that the current departmental publications acknowledgement statement is inserted. The authors will also acknowledge the study funding bodies and sponsor and may also include a publication specific statement as appropriate.

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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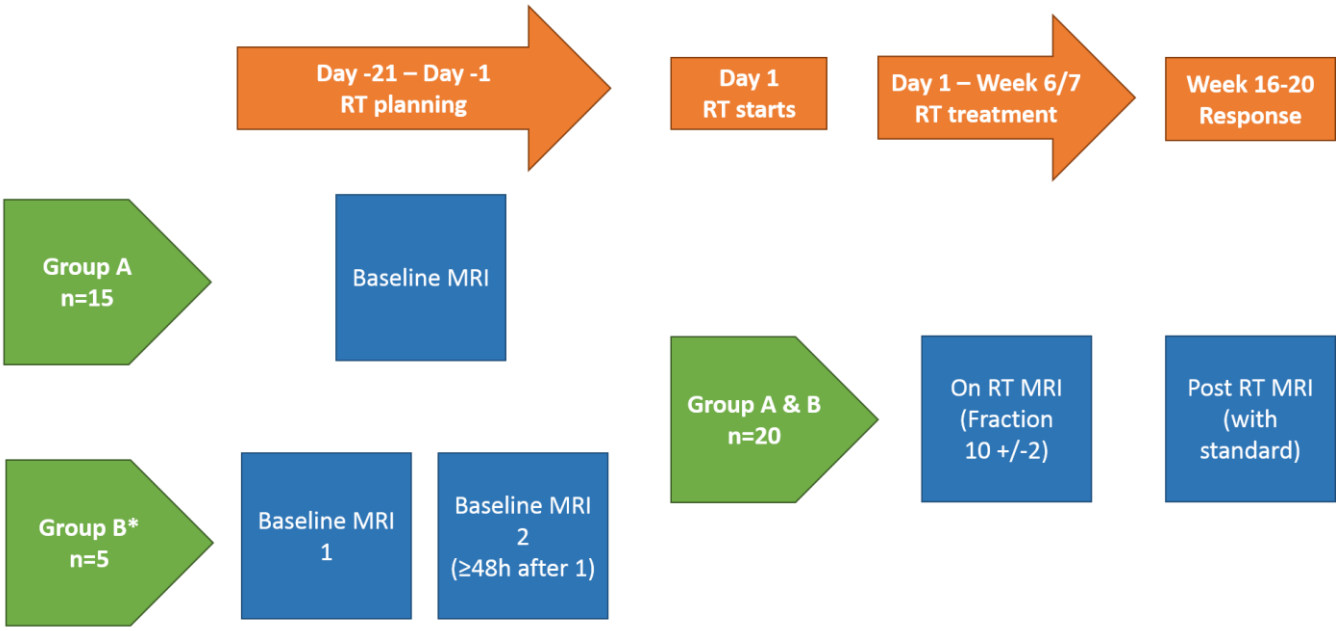
17. APPENDIX A: STUDY FLOW CHART



*5 patients will enter Group B where willing and able to undergo 2 scans prior to commencing RT

18. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee.