PhD Project Proposal

Funder details

Studentship funded by:	MRC Doctoral Training Programme

Project details

Project title:	Metabolic MRI of paediatric-type diffuse high grade glioma and its response to therapy *in vivo*

Supervisory team

Primary Supervisor:	Prof. Simon Robinson
Associate Supervisor(s):	Dr. Jessica Boult
Secondary Supervisor:	Prof. Chris Jones

Divisional affiliation

Primary Division:	Radiotherapy and Imaging
Primary Team:	Pre-Clinical MRI
Site:	Sutton

Project background

Paediatric-type diffuse high grade glioma (PDHGG), a malignant brain tumour, is a leading cause of tumour-related death in children and young adults. In the majority of cases median survival is only 9-18 months, with 2-year survival rates of less than 5% in patients with certain subtypes (1). Extensive genomic and epigenetic profiling has revealed distinct underlying biology in paediatric disease compared with histologically similar lesions in older adults, which differs by anatomical location. Amongst these, recurrent mutations in genes encoding histones H3.3 and H3.1 have been identified in around half of all PDHGGs, with H3 K27 alterations occurring in diffuse midline gliomas (DMG H3 K27-altered), and H3.3 G34R/V mutations arising in diffuse hemispheric gliomas (DHG H3 G34-mutant). PDHGGs lacking H3 and IDH1 mutations (PDHGG H3-wildtype, IDH-wildtype (wt)), are heterogeneous, with subgroups enriched for MYCN amplification and alterations in receptor tyrosine kinases (RTK) such as EGFR or PDGFRA (2,3). These alterations promote numerous oncogenic signalling pathways, including metabolic adaptations, that are revealing new therapeutic vulnerabilities for improved targeted treatments. Such metabolic reprogramming can also be leveraged by magnetic resonance imaging (MRI) approaches sensitive to tissue biochemistry, potentially providing much-needed early imaging biomarkers of PDHGG detection, delineation and therapeutic response.

MRI is routinely used for diagnosis and monitoring of paediatric brain tumours, however the infiltrative growth patterns of PDHGG can limit the efficiency of conventional MRI to fully delineate active disease *in situ*, and for early assessment of effective treatment response.

We have established metabolic MRI methods to characterise DMG H3 K27-altered, DHG H3 G34-mutant and H3 wt PDHGG xenografts derived from site-specific orthotopic implantation of patient-derived cells that retain the key genetic/epigenetic features (Figure 1). These models will underpin the pre-clinical work in this project.

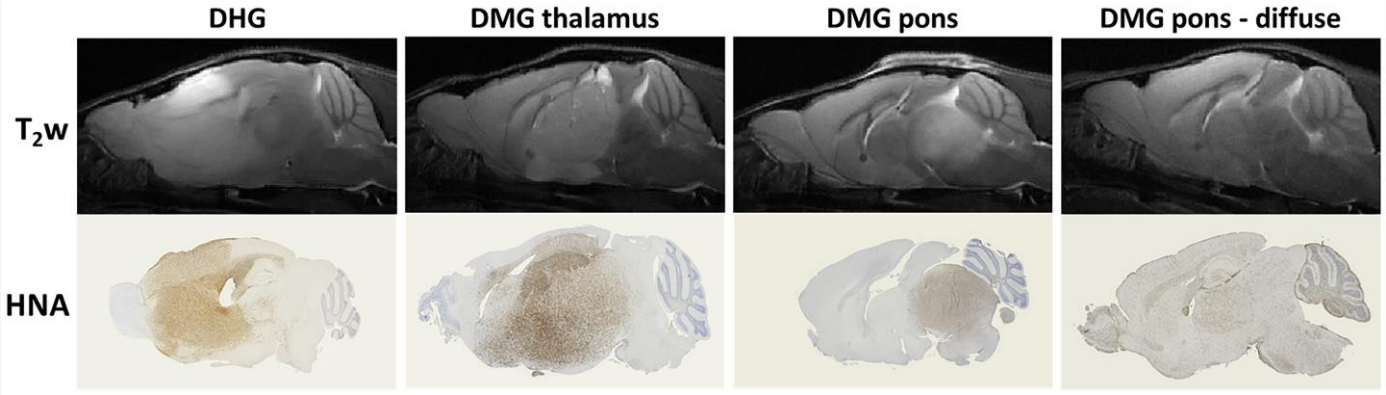

Figure 1. *T2-weighted (T2w) MRI and matched human nuclear antigen (HNA) immunohistochemistry, which stains human tumour cells, of orthotopic PDHGG xenografts derived from site specific injection of patient-derived tumour cells. Examples of tumours derived from diffuse hemispheric glioma (DHG) cells injected into the frontal lobe, thalamic diffuse midline glioma (DMG) cells injected into the thalamus, and brainstem DMG cells injected into the pons, including a brainstem tumour that grew with a very diffuse infiltrative pattern.*

Project aims

- Exploit metabolic MRI protocols to interrogate the detection and evolution of intracranial models of PDHGG and to correlate with histopathology.
- Utilise metabolic MRI methods to assess tumour response to treatments that modulate dysregulated tumour metabolism.
- Optimise targeted irradiation protocols for brain tumour treatment using the small animal radiation research platform (SARRP) and assess response to radiotherapy ± targeted therapies using multi-parametric and metabolic MRI.

Research proposal

It will be hosted within the Centre for Cancer Imaging (CCI) in Sutton, which provides a state of art, collaborative, multi-disciplinary pre-clinical research environment, with imaging and therapy equipment located adjacent to each other. The project will build upon a longstanding, productive collaboration between Prof. Simon Robinson's pre-clinical MRI team and the paediatric glioma team lead by Prof. Chris Jones.

Both established and novel orthotopically implanted models of PDHGG, grown in the specific location of the original tumour, will be propagated following protocols routinely used at the ICR. These may come from patient-derived stem cell cultures or from tissue harvested directly from patients. There will also be opportunities to exploit syngeneic models of PDHGG using cells isolated from tumours induced with mutations to model diffuse midline or hemispheric glioma in immunocompetent mice, propagated in the relevant region of the brain in strain-matched mice (4). Whilst these models are not derived from human tumours, they do allow for the study of the influence of the immune system in treatment response, particularly relevant in the context of radiation response.

Metabolic MR techniques which have been established on our dedicated pre-clinical horizontal bore MRI scanner will be exploited alongside conventional MRI imaging. These methods include:

i) Chemical exchange saturation transfer (CEST) MRI

CEST MRI can provide multiple and discrete contrasts relating to immobile (ssMT) and mobile (rNOE) macromolecules, as well as proteins (amides and amines), at high resolution, and is being used clinically to investigate metabolism in adult brain tumours (5). Our preliminary data demonstrate that CEST MRI provides clear delineation of well-defined orthotopic PDHGG xenografts, and has great potential to provide more sensitive imaging biomarkers for the early detection and delineation of diffuse PDHGG tumours than conventional anatomical imaging (Figure 2).

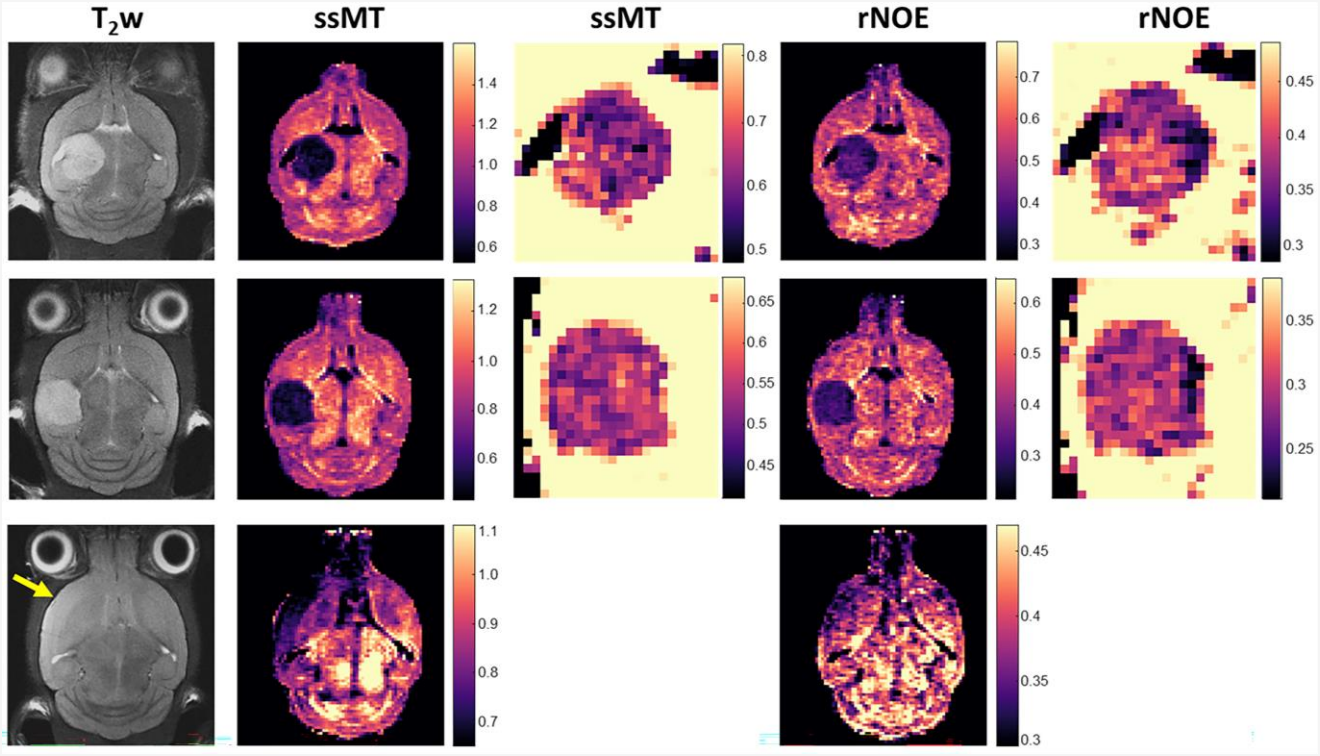

Figure 2. *T_2w MRI of three orthotopic PDHGG xenograft models alongside maps of CEST contrasts relating to immobile (ssMT) and mobile (rNOE) macromolecules, which show clear tumour delineation for two models (top and middle rows). Localised ssMT and rNOE maps scaled using a narrower dynamic range show the heterogeneous contrast present within these tumours. CEST maps of a diffusely growing tumour (bottom row) show more image contrast in the region of tumour growth (arrowed) than the anatomical T_2w image.*

In this project, longitudinal CEST MRI data will be acquired to map and quantify the evolving metabolic phenotype across orthotopic PDHGG models that encompass a range of molecular subtypes and tumour growth patterns, from expansive to diffusely infiltrative. CEST MRI will also be used to monitor PDHGG response to therapeutic strategies predicted to alter the macromolecular signature of tumours, e.g. radiotherapy, and to evaluate new metabolic interventions. For example, targeting of cholesterol synthesis, which has been identified as a metabolic vulnerability in H3 K27-altered DMG, with statins as part of combinatorial treatment being investigated in the Jones laboratory (6). Conventional histological correlates (e.g. human nuclear antigen (HNA) immunohistochemistry, H&E staining) to CEST contrast signal heterogeneity will be sought.

ii) Deuterium metabolic imaging (DMI)

Deuterium (^2H) is a MR visible isotope of hydrogen with low natural abundance. DMI is a MR spectroscopic imaging technique which, coupled with judicious choice of ^2H-labelled substrates, can inform on specific metabolic pathways *in vivo*. The metabolism of both adult brain tumour models *in vivo* and glioma patients has been investigated by DMI using [6,6-$^2H_2$]-glucose whose metabolism via glycolysis results in detectable labelling of lactate, and following entry of labelled pyruvate into the TCA cycle, detectable glutamine/glutamate (7,8).

We have established a novel *in vitro* ^2H MR spectroscopy assay to dynamically monitor the metabolism of deuterated substrates in live cells. Using this approach, and [6,6-$^2H_2$]-glucose, we have shown a significant reduction in glycolysis in *PIK3R1*-mutant PDHGG cells treated with the PI3K/mTOR inhibitor paxalisib, which was detected prior to any change in cell viability (Figure 3A). This project will utilise this method, and subsequently our established *in vivo* DMI methodology (Figure 3B), to further assess differential glycolytic responses to paxalisib across PDHGG models, and to evaluate response and resistance to other therapeutic agents whose mechanism of action elicits changes in tumour metabolism, for example MEK inhibitor trametinib. In addition, dordaviprone (ONC-201), which drives proteolysis of electron transport chain and TCA cycle proteins, thereby impairing tumour cell metabolism, inducing mitochondrial damage and rendering the tumour in a state of energy depletion, has shown clinical efficacy in DMG alone and in combination with paxalisib (9,10).

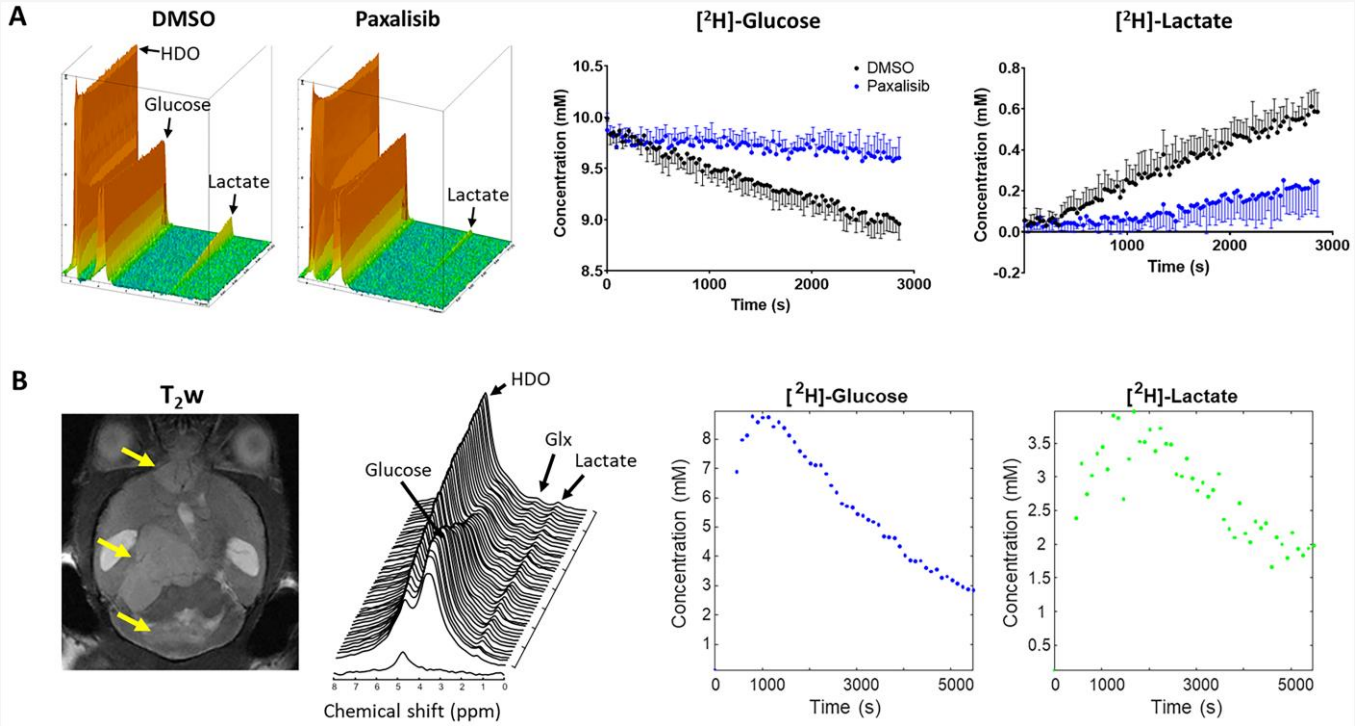

Figure 3. **A)** *A time series of dynamically acquired ^2H spectra, showing the evolution of the ^2H metabolites, acquired from PIK3R1-*mutant *PDHGG cells treated in vitro with DMSO or paxalisib for 72h. Peaks for semi-heavy water (HDO), [^2H]-glucose and [^2H]-lactate are indicated. Following calibration to mM concentrations, a clear reduction in both glucose consumption and lactate production was evident in paxalisib treated cells.* **B)** *T_2w MRI of a mouse brain showing multiple tumour nodes (arrowed) alongside dynamically acquired ^2H spectra acquired from the whole tumour-bearing brain before (1 spectrum) and after intravenous injection of [^2H]-glucose. Peaks for HDO, glucose, lactate and glutamine/glutamate (Glx) are indicated; note the rapid increase in the magnitude of the peaks after injection. The metabolites were quantified at each timepoint and their evolution over time can be evaluated.*

Opportunities to explore alternative deuterated metabolic tracers to investigate other potentially targetable metabolic vulnerabilities in PDHGG will also be expoited. DMI has revealed distinct contrast between orthotopic rat gliomas and normal-appearing brain after [$^2H_3$]-acetate infusion, showing higher acetate levels and lower acetate oxidation in the tumour (7). Our ^2H MRS *in vitro* assay and *in vivo* DMI will be used to investigate whether [$^2H_3$]-acetate uptake can i) reveal differential metabolism between PDHGG models, and ii) assess tumour response to paxalisib and/or dordaviprone.

Frontline therapy for patients over 3 years old with PDHGG includes radiotherapy, in combination with surgery and chemotherapy where possible, or alone for DMGs arising in the brainstem. Tumour irradiation in this project will be performed using the small animal radiation research platform (SARRP) within the CCI, which replicates modern clinical radiotherapy for conformal treatment of rodent tumour models. The SARRP has an integrated CT scanner for treatment planning, with the potential to import and fuse MRI images, enabling accurate irradiation of individual tumours with 0.5mm precision.

Through a new collaboration with Prof. Josephine Bunch at National Physical Laboratory, we will use correlative mass spectrometry imaging (MSI) of MRI-aligned tissue sections to provide more definitive identification and spatial distribution of the endogenous metabolites contributing to the CEST contrast and DMI data.

Clinical translation of these methods will be explored via the London Collaborative Ultra-high field System (LoCUS) facility, a clinical 7T MRI system located within St. Thomas Hospital, for which the ICR is the lead oncology partner. LoCUS provides a unique opportunity to exploit the greatly increased sensitivity to physiological and metabolic processes afforded by ultra-high field MRI in adult and paediatric patient studies, and we will work with clinical colleagues to develop and apply CEST MRI and DMI on this system, towards investigating tumour metabolism and treatment response in PDHGG patients.

Literature references

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Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:

BSc or equivalent in either Biological Sciences or Physics/Engineering

Intended learning outcomes:

- Competency in the maintenance of cultures of primary cells taken from surgical specimens, grown either as monolayers or three-dimensional neurospheres.
- Secure a Home Office licence, become a responsible licensee and become proficient in the propagation of orthotopic brain tumour models *in vivo*.
- Development and application of non-invasive, clinically translatable MRI modalities for the preclinical assessment of brain tumour metabolism and changes in response to therapeutic intervention *in vivo*.
- Gain an appreciation of clinical imaging approaches for the assessment of paediatric brain tumours.
- Develop strong and confident communication skills through regular presentations of their work at lab meetings, departmental seminars and report writing.

- Training will be provided within a stimulating research environment in which many projects are of a multi-disciplinary or collaborative nature, providing an insight into a wide range of imaging techniques and expertise.

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☒ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science