The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Advanced functional MRI for the characterisation of paediatric glioma and its response to therapy**

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Simon Robinson
Associate Supervisor(s):	Dr Jessica Boult, Dr Yann Jamin
Backup Supervisor: (must have IRS status)	Prof Chris Jones
Lead contact person for the project:	Dr Simon Robinson

DIVISIONAL AFFILIATION	
Primary Division:	Radiotherapy and Imaging
Primary Team:	Magnetic Resonance Imaging

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Paediatric glioblastoma (pGBM) and diffuse intrinsic pontine glioma (DIPG) remain leading causes of tumour-related morbidity and mortality in children and young adults, with median survival of 12-15 months (Ostrom et al., 2014). The clinical and molecular differences observed in paediatric disease compared with histologically similar lesions in older adults has revealed distinct underlying biology, which differs by anatomical location (Jones and Baker, 2014). Recurrent mutations in genes encoding histones H3.3 and H3.1 have been identified, with H3.3 G34R/V expressed in hemispheric tumours often co-segregating with TP53 mutations, and K27M variants appearing in midline tumours including DIPG (Mackay et al., 2017).

Current primary treatment options are based on adult protocols. For surgically-accessible tumours, such as those arising in the cerebral hemispheres, surgical resection is followed by temozolomide and irradiation, whilst for DIPG, where the site and diffuse pattern of growth precludes surgery, irradiation is the frontline option. Following recent extensive genomic and epigenetic profiling, orthotopic models that accurately recapitulate these tumours are being developed, and novel therapies are being sought that target the specific molecular pathways dysregulated in pGBM/DIPG (Figures 1&2). The evaluation of such therapies in these models demands non-invasive imaging strategies to accurately monitor tumour progression and response *in vivo.*

Magnetic resonance imaging (MRI) is routinely used for diagnosis of paediatric brain tumours. However, areas of diffuse growth in pGBM/DIPG do not typically enhance following administration of a gadolinium-based contrast agent (GBCA), a consequence of an intact blood-brain barrier (BBB) precluding contrast agent extravasation. Accurate assessment of treatment response can also be hindered by short-term changes in vascular permeability, damage to myelin sheaths, and inflammation following standard-of-care radiotherapy and chemotherapy. Consequently, more advanced non-invasive imaging methods are required to provide biomarkers that accurately inform on the extent, progression and response/resistance to treatment in pGBM/DIPG *in vivo*, and which may also provide insight into changes in metabolism and molecular signalling pathway alterations.

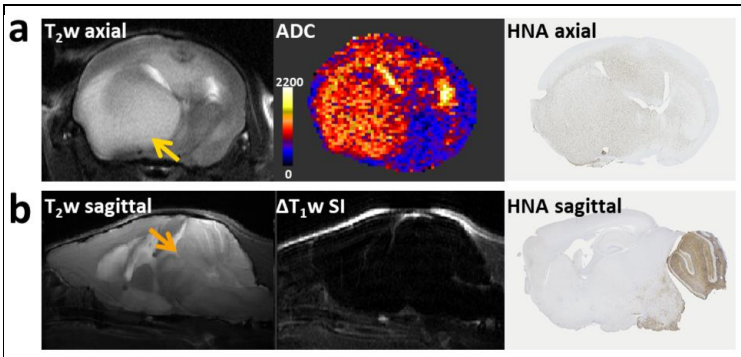

Figure 1. a) A pGBM xenograft propagated in the frontal lobe is hyperintense in a T_2-weighted MR image (T_2w) and exhibits elevated apparent diffusion coefficient (ADC, $x10^{-6}$ mm^2/s), attributed to oedema. Human nuclear antigen (HNA) immunohistochemistry shows a diffuse tumour. **b)** A patient-derived DIPG xenograft propagated in the pons presents as a diffusely hyperintense lesion in the pons and cerebellum in a T_2w image. No signal change is observed by T_1w MRI following GBCA administration (ΔT_1w SI) indicating an intact BBB (note bright regions of enhancement in areas around brain). HNA staining demonstrates a diffuse but hypercellular tumour in the cerebellum and pons.

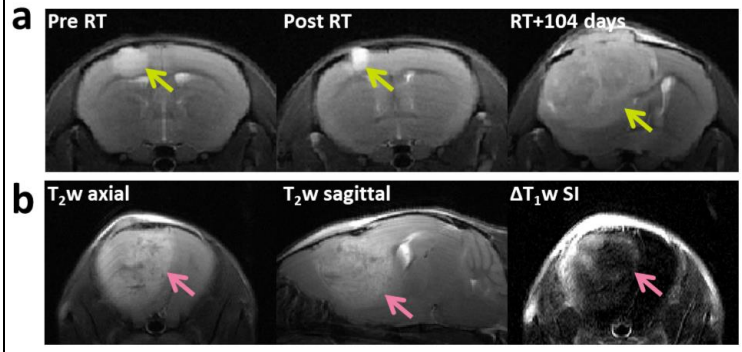

Figure 2. a) T_2w MRI shows a volume reduction in a cell line derived pGBM xenograft following 6x2Gy irradiation targeted to the brain using the small animal radiation research platform (SARRP) in combination with a radio-sensitising drug. MRI was also used to track subsequent tumour regrowth. **b)** A syngeneic mouse-derived pGBM model grown in an immunocompetent host presents as a relatively well defined mass by T_2w MRI. Heterogeneous signal enhancement is observed in the tumour on T_1w MRI following GBCA administration, demonstrating that the BBB is compromised in at least some areas of the tumour.

PROJECT AIMS

- Implement advanced multi-parametric MRI protocols to interrogate the growth patterns of intracranial models of paediatric GBM and DIPG and to correlate with histopathology.
- To optimise targeted irradiation protocols for brain tumour treatment using the small animal radiation research platform (SARRP) and assess response to radiotherapy using multi-parametric MRI.
- Design rational MRI-embedded combination treatment protocols encompassing irradiation, novel targeted therapeutics and, if appropriate, standard chemotherapeutics.

RESEARCH PROPOSAL

This project will develop, optimise and apply multi-parametric MRI strategies to interrogate the evolving phenotype of orthotopic pGBM and DIPG models, and to assess differential treatment response to both standard-of-care treatment regimens and rational therapeutics targeted towards the pathways dysregulated in pGBM/DIPG. It will be hosted within the Centre for Cancer Imaging (CCI) in Sutton, which provides a state of art, collaborative, multi-disciplinary research environment, with imaging and therapy equipment located adjacent to each other. The project will allow for close collaboration with scientists from the prestigious CR-UK Cancer Therapeutics Unit, and builds upon a longstanding, productive collaboration between Dr. Simon Robinson's preclinical MRI team and the paediatric glioma team lead by Prof. Chris Jones.

Both established and novel orthotopically implanted models of paediatric glioma, grown in the specific location of the original tumour, will be propagated following protocols routinely used at the ICR. These will primarily come from patient-derived stem cell cultures, but also from tissue harvested directly from patients. There will also be opportunities to exploit syngeneic models of pGBM using cells isolated from tumours induced with mutations to

model DIPG or hemispheric glioblastoma in immunocompetent mice (RCAS/ACVR1R206H/PDGFA/H3.1K27M/p53 loss (Hoeman et al., 2019) or NRAS/shP53/shATRX/H3.3G34R (Nunez et al., 2015), respectively), propagated in the relevant region of the brain in strain-matched mice. Whilst these models are not derived from human tumours, they do allow for study of the influence of the immune system in treatment response, particularly relevant in the context of radiation response.

Where possible, tumour establishment and growth will be monitored by bioluminescent imaging (BLI). However, in new models and those in which luciferase expression is not achievable, anatomical MRI will be used to monitor tumour growth and treatment response (Figures 1&2). Diffuse infiltrative tumour growth is observed in the majority of pGBM tumours clinically and has been recapitulated in patient-derived models propagated at the ICR (Figure 1). More advanced MRI techniques will be optimised and exploited to more accurately delineate, and inform on the tumour structure and function. These will include endogenous contrast methods to assess water diffusivity (a surrogate biomarker of cellularity and marker of oedema, often associated with invasion. Figure 1a), mapping of MRI relaxation times T_1 and T_2, and protocols incorporating contrast agents that either remain within the vasculature (ultrasmall superparamagnetic iron oxide (USPIO) particles) or leak out of tumour vessels (GBCA, Figures 1b&2b) (Boult et al., 2016). The utility of contrast agent free arterial spin labelling (ASL), which is increasingly being used to quantify blood flow in adult brain tumours (Warmuth et al., 2003), and has shown potential to delineate infiltrating brain tumour regions preclinically (Vallatos et al., 2019), will be exploited. In addition, amide proton transfer (APT), a chemical exchange saturation transfer (CEST) method in which the signal is based on the higher concentration of endogenous proteins and peptides typically present in high grade brain tumour tissue, will be investigated. APT may enable better identification of tumour boundaries, differentiation between tumour and off-target treatment effects, and predict response to therapy (Ma et al., 2016). These methods have the potential to be informative in native tumours, but we also aim to evaluate their utility in assessing early and long-term response to treatment.

Standard-of-care treatment for patients with pGBM and DIPG over 3 years old includes radiotherapy, in combination with surgery and chemotherapy in the case of hemispheric tumours, or alone for DIPG. Therefore, we will seek to combine and compare the targeting of novel therapeutic targets with these standard treatment regimens in our studies. For tumour irradiation, the SARRP system within the CCI, which replicates modern clinical radiotherapy for conformal treatment of rodent tumour models, will be used (Figure 1a). 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. New dosimetry and protocols for intensity modulated beam delivery are under development in Prof. Uwe Oelfke's laboratory to establish more accurate dose targeting (Reinhart et al., 2018), significantly reducing the amount of tissue exposed to radiation and hence the likelihood and severity of normal tissue toxicity.

Rational novel therapeutic targets for the selected models will be guided by genomic and methylation profiling and *in vitro* drug screening against a panel of ~400 approved chemotherapeutics and small molecules (Mackay et al., 2018). Assessment of 21 patient-derived cultures has established that H3.3 G34R cells appear differentially sensitive to agents targeting the proteasome, whilst H3.3 K27M cells are responsive to crizotinib; cells with sensitising and resistance mutations to PDGFRA inhibition were also identified.

Histopathological assessment, including qualification of MRI biomarkers and evaluation of heterogeneity, will be paramount. Microscopic assessment of morphology, vascularisation, vascular perfusion, necrosis and infiltration will be performed, and credentialed in respect of the WHO classification of the human disease. A range of immunohistochemical markers used in routine differential diagnosis will be employed (GFAP, desmin) in addition to markers of DNA damage/repair in response to irradiation (γH2AX, RAD51), cell death and proliferation (Caspase 3, Ki67), and immune infiltrate (F4/80, CD8, FOX3P, S100).

LITERATURE REFERENCES

BOULT, J. K., BORRI, M., JURY, A., POPOV, S., BOX, G., PERRYMAN, L., ECCLES, S. A., JONES, C. & ROBINSON, S. P. 2016. Investigating intracranial tumour growth patterns with multiparametric MRI incorporating Gd-DTPA and USPIO-enhanced imaging. *NMR Biomed,* 29**,** 1608-1617.

HOEMAN, C. M., CORDERO, F. J., HU, G., MISURACA, K., ROMERO, M. M., CARDONA, H. J., NAZARIAN, J., HASHIZUME, R., MCLENDON, R., YU, P., PROCISSI, D., GADD, S. & BECHER, O. J. 2019. ACVR1 R206H cooperates with H3.1K27M in promoting diffuse intrinsic pontine glioma pathogenesis. *Nat Commun,* 10**,** 1023.

JONES, C. & BAKER, S. J. 2014. Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma. *Nat Rev Cancer,* 14**,** 651-661.

MA, B., BLAKELEY, J. O., HONG, X., ZHANG, H., JIANG, S., BLAIR, L., ZHANG, Y., HEO, H. Y., ZHANG, M., VAN ZIJL, P. C. & ZHOU, J. 2016. Applying amide proton transfer-weighted MRI to distinguish pseudoprogression from true progression in malignant gliomas. *J Magn Reson Imaging,* 44**,** 456-62.

MACKAY, A., BURFORD, A., CARVALHO, D., IZQUIERDO, E., FAZAL-SALOM, J., TAYLOR, K. R., BJERKE, L., CLARKE, M., VINCI, M., NANDHABALAN, M., TEMELSO, S., POPOV, S., MOLINARI, V., RAMAN, P., WAANDERS, A. J., HAN, H. J., GUPTA, S., MARSHALL, L., ZACHAROULIS, S., VAIDYA, S., MANDEVILLE, H. C., BRIDGES, L. R., MARTIN, A. J., AL-SARRAJ, S., CHANDLER, C., NG, H. K., LI, X., MU, K., TRABELSI, S., BRAHIM, D. H., KISLJAKOV, A. N., KONOVALOV, D. M., MOORE, A. S., CARCABOSO, A. M., SUNOL, M., DE TORRES, C., CRUZ, O., MORA, J., SHATS, L. I., STAVALE, J. N., BIDINOTTO, L. T., REIS, R. M., ENTZ-WERLE, N., FARRELL, M., CRYAN, J., CRIMMINS, D., CAIRD, J., PEARS, J., MONJE, M., DEBILY, M. A., CASTEL, D., GRILL, J., HAWKINS, C., NIKBAKHT, H., JABADO, N., BAKER, S. J., PFISTER, S. M., JONES, D. T. W., FOULADI, M., VON BUEREN, A. O., BAUDIS, M., RESNICK, A. & JONES, C. 2017. Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell,* 32**,** 520-537 e5.

MACKAY, A., MOLINARI, V., CARVALHO, D., PEMBERTON, H., TEMELSO, S., BURFORD, A., CLARKE, M., FOFANA, M., BOULT, J., IZQUIERDO, E., TAYLOR, K., BJERKE, L., FAZAL SALOM, J., KESSLER, K., ROGERS, R., CHANDLER, C., ZEBIAN, B., MARTIN, A., STAPLETON, S., HETTIGE, S., MARSHALL, L., CARCELLER, F., MANDEVILLE, H., VAIDYA, S., BRIDGES, L. R., AL-SARRAJ, S., PEARS, J., MASTRONUZZI, A., CARAI, A., DEL BUFALO, F., DE TORRES, C., SUNOL, M., CRUZ, O., MORA, J., MOORE, A., ROBINSON, S., LORD, C., CARCABOSO, A. M., VINCI, M. & JONES, C. 2018 Drug Screening Linked to Molecular Profiling Identifies Novel Dependencies in Patient-Derived Primary Cultures of Paediatric High Grade Glioma and DIPG. *Neuro Oncol. ,* 20**,** i93–i94.

NUNEZ, F. J., MENDEZ, F. M., KOSCHMANN, C., CALINESCU, A., DZAMAN, M., LOWENSTEIN, P. R. & CASTRO, M. G. 2015. A Novel Mouse Model of Young Adult Glioblastoma: In Vivo Expression of Mutated Idh1 (R132h) Gene Using the Sleeping Beauty Transposase System. *Neuro-Oncology,* 17**,** 11-11.

OSTROM, Q. T., GITTLEMAN, H., LIAO, P., ROUSE, C., CHEN, Y., DOWLING, J., WOLINSKY, Y., KRUCHKO, C. & BARNHOLTZ-SLOAN, J. 2014. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007–2011. *Neuro Oncol,* 16**,** iv1-iv63.

REINHART, A. M., NILL, S. & OELFKE, U. 2018. mIMRT - Bringing small animal irradiations closer to clinical standards. *Radiotherapy and Oncology,* 127**,** S319-S319.

VALLATOS, A., AL-MUBARAK, H. F. I., BIRCH, J. L., GALLLAGHER, L., MULLIN, J. M., GILMOUR, L., HOLMES, W. M. & CHALMERS, A. J. 2019. Quantitative histopathologic assessment of perfusion MRI as a marker of glioblastoma cell infiltration in and beyond the peritumoral edema region. *J Magn Reson Imaging,* 50**,** 529-540.

WARMUTH, C., GUNTHER, M. & ZIMMER, C. 2003. Quantification of blood flow in brain tumors: comparison of arterial spin labeling and dynamic susceptibility-weighted contrast-enhanced MR imaging. *Radiology,* 228**,** 523-32.

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: e.g. BSc or equivalent in specific subject area(s)	BSc or equivalent in either Physics, Biological Sciences or 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 tumours and therapeutic response *in vivo*. - Establish and implement targeted irradiation protocols for the treatment of orthotopic brain tumour models *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 ☐ Other (provide details)
Keywords:	**1. Magnetic resonance imaging**
	2. Brain tumours
	3. Childhood cancer
	4. Radiotherapy
	5. Cancer therapeutics