The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	The Institute of Cancer Research (ICR)
PROJECT DETAILS	
Project Title	Use of PET imaging to monitor immune cell fitness and responses induced by suppression of the PI3K/AKT pathway in glioblastoma.
Short Project Title	**PET imaging to monitor immune cell fitness post-targeted treatment in GBM**
SUPERVISORY TEAM	
Primary Supervisor	Dr Gabriela Kramer-Marek
Associate Supervisor:	Dr Juanita Lopez
Secondary Supervisor	Professor Udai Banerji
DIVISIONAL AFFILIATION	
Primary Division	Radiotherapy and Imaging
Primary Team	Preclinical Molecular Imaging

BACKGROUND TO THE PROJECT

Glioblastoma (GBM) is the most common primary malignant brain tumour in adults and is associated with an extremely aggressive clinical course and poor prognosis (1). The median progression-free survival in primary GBM is 6.9 months, and median overall survival is 14.6 months with standard-of-care surgery and combined radiation with temozolomide chemotherapy (2). Despite such intensive therapeutic regimens, almost all patients with GBM eventually relapse, mainly due to diffuse microscopic infiltration of tumour cells into the surrounding brain parenchyma. Single-cell RNA sequencing revealed that multiple subtypes could reside within the same tumour, indicating heterogeneity of GBM at the molecular and cellular levels (3). Consequently, this phenomenon not only limits the efficacy of multimodal treatment combinations, but triggers activation of resistance mechanisms that further impede complete tumour eradication (4).

Therefore, identifying and targeting cooperative pathways within a particular GBM subtype is likely to lead to new treatment paradigms that will ultimately allow for more efficient tumour suppression and durable responses.

AKT is the most crucial proximal node downstream of the receptor tyrosine kinase-PI3K complex regulating cell growth, metabolism, proliferation, and survival. Of note, the main negative regulator of AKT, the tumour suppressor gene PTEN, is inactive in over 50% of GBMs, which leads to elevated activity of AKT (5). Despite intensive research, inhibitors

targeting this signalling cascade have so far yielded little therapeutic value in GBM patients. Nevertheless, recent clinical trials and pre-clinical studies have demonstrated that inhibition of the PI3K/AKT/mTOR pathway may result in augmenting tumour immunosurveillance by preventing activation of immunosuppressive pathways and enhancing anti-tumour immune-intrinsic properties. **These findings suggest that AKT should be re-evaluated as a potential target for combination with immune checkpoint inhibitors (ICPIs) in GBM patients.**

PROJECT AIMS

This PhD project aims to discover novel imaging biomarkers of GBM, which can be translated into the clinic for treatment management.

Objective 1. To investigate the response to PI3K/AKT inhibitor (e.g. ipatasertib) alone and in combination with anti-PD-L1 mAb (e.g. atezolizumab) in PTEN deficient and PTEN wild type GBM models (2D and 3D) as well as their co-cultures with astrocytes and GBM cancer associated fibroblasts (CAFs).

Objective 2. To assess by PET imaging whether PI3K/AKT inhibition *in vivo* modifies tumour microenvironment (TME) toward promotion of an effective anti-cancer immune response.

Objective 3. To determine whether targeting PI3K/AKT signalling in combination with ICPIs *in vivo* enhance the immune cell infiltration prolonging the survival. To evaluate whether PET imaging biomarkers will quantitatively measure tumour response induced by these therapeutic intervention.

RESEARCH PROPOSAL

Rationale:

Recently, ICPIs targeting programmed cell death 1 receptor (PD-1; e.g. nivolumab, pembrolizumab) and its ligand (PD-L1; e.g. durvalumab, atezolizumab) have been approved and licensed in multiple tumour types (6). Data from clinical trials show that PD-L1 expression, measured by tumour proportion score (TPS) or combined pathological score (CPS), is a potential biomarker to guide selection of patients who could benefit from ICPIs. Regrettably, for GBM patients only modest and unpredictable responses have been reported so far. This is most likely due to a relatively immune-depleted ("cold") GBM microenvironment characterised by: i) absence of tumour-infiltrating lymphocytes (TILs); ii) exhaustion of cytotoxic T-lymphocytes (CTLs); and iii) high level of immunosuppressive cytokines (e.g. TGFβ, IL-10) that inhibit T-cell activity (7). Encouragingly, recent findings have demonstrated that pronounced infiltration of pre-existing $CD8^+$ CTLs into the tumour microenvironment (TME) can render GBM more responsive to ICPIs. Therefore, in a clinical context, a desirable outcome would be to restore the immunological environment of GBM to improve the response of these tumours to ICPIs. We hypothesise that **targeting PI3K/AKT with brain penetrable inhibitor** (e.g. ipatasertib) not only directly impact cancer cells but also **has the capacity to affect immune cell effector functions and to modulate immune TME improving the efficacy of ICPIs.** Furthermore, we **postulate that PET imaging will non-invasively measure these changes.**

Last but not least, given previous reports demonstrating that apart from the tumour cell-intrinsic mechanisms that cause constitutive PI3K/AKT pathway activation, also factors secreted from CAFs can maintain this pathway, we will assess whether a transmembrane fibroblast activation protein (FAP) can affect the GBM cells sensitivity to PI3K/AKT inhibition. The role of FAP within the glioma microenvironment is still unclear. However, it has been recently revealed that **subpopulations of GBM and stromal cells with mesenchymal features overexpress FAP** (8). Now it is also well recognised that **GBMs with mesenchymal features (FAP^+) have elevated levels of PD-L1 and highest presence of TAM, $CD8^+$, $CD3^+$ and $FOXP3^+$T cells**, which suggests they may be more immunoreactive in nature, and therefore, more amenable to immunotherapy (9). However, little is known about the relationship between FAP^+ mesenchymal GBM cells and PI3K/AKT signalling in this complex phenotype.

Approach:

- GBM cell lines and patient-derived neurospheres with varying PTEN status will be used. Initially, we will assess whether the anti-PD-L1 treatment in combination with e.g. ipatasertib directly affects PD-L1 present on cancer cells (i.e. independently of T cells). Protein and/or mRNA expression level of selected proteins (e.g. AKT/pAKT, PRAS40/pPRAS40, S6/pS6 and PD-L1) will be measured by Western blot (WB), qRT-PCR and flow cytometry. In addition, the protein expression level of FAP will be evaluated in all cell lines with confirmed mesenchymal phenotype.

- The response to ipatasertib *in vivo* will be demonstrated by the survival time of mice bearing e.g. the orthotopic xenograft and syngeneic tumours. The growth of brain tumours post-cells implantation will be monitored by MRI/BLI. On day 8-10 post-implantation, mice will be randomised into the treatment groups. Tumour growth/inhibition will be monitored every second day by MRI and BLI imaging. Post-treatment we will evaluate tumour associated macrophages (TAM) as they can compromise up to 50% of the tumour mass in GBM and play an essential role in inducing drug resistance (10). Furthermore, we will measure the level of activated NK cells in the tumour. We will also assess whether inhibition of PI3K/AKT will suppress the production of proinflammatory cytokines secreted by immune cells. Multiplex immunostaining (IHC) and multiparameter flow cytometry will be conducted.
- To investigate whether PI3K/AKT inhibition can generate immunological memory, the response to the PI3K/AKT inhibitor in combination with ICPIs will be determined in syngeneic GBM models. The growth of brain tumours post-cells implantation will be monitored by MRI or BLI (where possible). On day 8-10 post-implantation, mice will be randomised into the treatment groups. During the treatment MRI or BLI will be performed every second day to monitor tumour response, defined by tumour growth inhibition in the presence of continued therapy. In addition, before treatment and on day 2 post-treatment initiation PET/CT using **^{18}F-FPIA**, **^{18}F-AlF-FAPI-74** and/or e.g. **^{68}Ga-DOTA-Affibody$_{PD-L1}$** will be acquired and data correlated with *ex vivo* methods.
  PI3K/AKT signalling is associated with the fatty acid metabolism and acetate contributes to over half of oxidative activity in GBM tumours, while glucose contributes to only a third. Along this line, **we will use** ^{18}F-fluoropivalate (**^{18}F-FPIA**) **to investigate metabolic reprogramming as a consequence of PI3K/AKT inhibition *in vivo***. Furthermore, we will evaluate the use of **^{18}F-AlF-FAPI-74** imaging: i) **to better understand the relationship between FAP protein** expression **and the immune suppression in GBM tumours** and, ii) to facilitate design of novel combinatorial immune therapeutic paradigms that can restore and induce robust tumour immunity. Ultimately, the **^{68}Ga-DOTA-Affibody$_{PD-L1}$, for example, will allow us to determine the baseline expression level of PD-L1** and monitor its dynamic changes post-treatment within the brain tumours.
  It is well recognised now that T cell 'metabolic fitness' is central to effective anti-tumour immunity, and is modulated by both the tumour nutrient microenvironment and immune checkpoints. Therefore, we will also conduct transcriptome analysis to examine the changes in metabolic gene profiles and correlate it with radiomic features. Furthermore, we will investigate the local tumour control and immunological effects (numbers and functional activation/exhaustion state of tumour infiltrating cells). In particular, we will measure cytokine and chemokine concentrations (e.g. TNF-α, IFN-γ, IL-1β) as well as numbers and functional activation/exhaustion state of different subpopulations of T-cells, Tregs and NK cells using multicolour flow cytometry, ELISA and IHC.

Outcome:

- We will establish a combination treatment strategy that will stimulate the immune response and break down the barriers of TME in conjunction with immunotherapies leading to the improvement of overall GBM patient's survival.
- We will have optimised imaging biomarkers that will predict the efficacy of these therapeutic regimens and guide dosing of individual drugs.

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LITERATURE REFERENCES

References:

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10. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nat Neurosci* 2016;**19**(1):20-7 doi 10.1038/nn.4185.

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	Candidates must have a first class or upper second class honours BSc Honours/MSc in in Medicine or Biological Sciences. An interest in immunology as well as a willingness to engage in the biology aspects of the project and to learn the associated techniques, all are essential. Evidence demonstrating such interests would be helpful.
Intended learning outcomes:	The student will learn: - The background to *in vitro* and *in vivo* assessment of novel PET radioligands - PET/MRI/BLI image acquisition and analysis - The role of immuno-oncology agents in glioblastoma models - Assessment of immune cell infiltrates by immunohistochemistry, flow cytometry, ELISA - Assessment of TME using molecular techniques

ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☒ Medicine
Keywords	1. Glioblastoma (GBM)
	2. PET imaging
	3. PI3K/AKT inhibition
	4. Fibroblast Activation Protein (FAP)
	5. Immuno-Oncology
	6. Immune Checkpoint Inhibitors