ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	Brain Tumour Research Centre of Excellence Grant

Project details

Project title:	**Photoimmunotherapy targeting of paediatric-type diffuse high-grade glioma (PDHGG)**

Supervisory team

Primary Supervisor:	**Dr Gabriela Kramer-Marek**
Associate Supervisor(s):	Dr Chiara Da Pieve
Secondary Supervisor:	Prof Chris Jones

Divisional affiliation

Primary Division(s):	Division of Radiotherapy and Imaging Division of Molecular Pathology
Primary Team(s):	Preclinical Molecular Imaging Glioma Team
Site:	Sutton

Project background

Paediatric-type diffuse high-grade glioma (PDHGG) are a collection of brain tumours in children and young adults with an extremely poor clinical outcome. For the vast majority of these tumours, the median survival is only 9-18 months, with 2-year survival rates of less than 5% of patients with certain subtypes. Much of the historical failure to improve survival in these patients stemmed from a lack of understanding of the biological differences with similar-looking tumours in adults, and within the diverse spectrum of what we call a 'high grade glioma' in the younger population. **The remarkable discoveries of PDHGG harbouring driving alterations in genes** previously unknown to be related to cancer, such as **the oncohistone H3 mutations and somatic ACVR1 mutations**, alongside exquisite transcriptional dependencies related to the stalled developmental origins of these tumours, has highlighted the necessity of developing bespoke and novel therapeutic strategies. With the international CONNECT consortium of paediatric neuro-oncology centres, **Prof Chris Jones has recently established a PDHGG Centre of Excellence**, to provide the resource and focus on screening hypothesis-driven concepts in well-characterised disease models with a view to generating robust preclinical data packages suitable for rapid clinical translation.

Photoimmunotherapy (PIT) is a light-mediated therapeutic approach in which a photosensitiser is conjugated to a highly specific targeting vector. When the conjugate is bound to antigen-presenting cancer cells, exposure to near-infrared (NIR) light leads to the disruption of cell membrane, followed by subsequent cell death, antigen release and recruitment of immune cells upon binding to pattern-recognition receptors.

Dr Gabriela Kramer-Marek's group has previously investigated this approach using EGFR-specific affibody molecule conjugated to IR700 ($Z_{EGFR:03115}$-IR700). This has been successfully applied to orthotopic adult glioblastoma models in vivo, promoting a therapeutic response with necrotic cell death and activation of T cells as early as several hours post-irradiation. **We now intend to apply this therapeutic approach to relevant targets and models in PDHGG**.

Project aims

- **To investigate** $Z_{EGFR:03115}$-IR700-PIT in EGFR-dependent PDHGG models *in vitro*.
- **To develop** and **characterise** novel light-activated conjugates directed against other cell surface receptors of relevance in PDHGG such as PDGFRA, ALK2, and GD2.
- **To determine** the efficacy and immune response to IR700-labelled PIT conjugates in appropriate PDX and syngeneic PDHGG models *in vivo.*

Research proposal

Aim 1: *To investigate $Z_{EGFR:03115}$-IR700-PIT in EGFR-dependent PDHGG models in vitro.*

We will first explore the use of the extant $Z_{EGFR:03115}$-IR700 conjugate in specific, highly infiltrative PDHGG subtypes driven by EGFR namely gliomatosis cerebri (GC), multi-lobar tumours recently characterised via a collaborative pan-European study, and *DMG-EGFR*, midline tumours that are enriched for bithalamic glioma. **We hypothesise that distinct *EGFR* mutations in tumours** arising in both anatomical compartments, rare in PDHGG without multi-lobar/bithalamic patterns of spread, **confer an enhanced infiltrative capacity**.

Firstly, we will assess the $Z_{EGFR:03115}$-IR700 specificity of binding using a panel of human and murine PDHGG cell lines (flow cytometry; confocal microscopy). The response to $Z_{EGFR:03115}$-IR700-PIT *in vitro* will be tested using 2D mono-layer cell cultures and 3D spheroids. Cell death will be assessed by luminescent assay, alongside the release of damage-associated molecular patterns (DAMPs) using immunoblotting, immunofluorescence and ELISA assays. Secondly, to verify whether $Z_{EGFR:03115}$-IR700-PIT induces pro-immunogenic cell death, we will perform dendritic cell (DC) maturation assays using supernatants from treated tumour cells (e.g., CD40, CD80, CD86 and HLA-DR expression on DC and IP-10, IFN-γ, IL-1β, TNF-α and IL-6 in the supernatant).

Aim 2: *To develop and characterise novel light-activated conjugates directed against other cell surface receptors of relevance in PDHGG such as PDGFRA, ALK2, and GD2.*

We will conjugate IR700 to the clinical grade specific monoclonal antibodies or affibody molecules (collaboration with AffibodyAB, Sweden). Following conjugation optimisation, the affinity and specificity of binding for each probe will be assessed against positive and negative cell lines (flow cytometry and confocal microscopy).

Furthermore, we will evaluate the suitability of the probes to target the antigen of interest *in vivo* using syngeneic mouse models of *PDHGG* and IVIS-Spectrum small animal imaging system. In iterative experiments we will determine timing for administration of the conjugates, tumour-to-background fluorescent intensity, and the biodistribution of fluorescence in different tissues. The imaging data will be also correlated with histopathology being a gold-standard.

Aim 3: *To determine the efficacy and immune response of IR700-labelled PIT conjugates in appropriate PDX and syngeneic PDHGG models in vivo.*

We have both syngeneic, immune competent models of H3-mutant and wild-type PDHGG derived from genetically engineered *de novo* mouse tumours, and patient-derived models in an immunocompromised background. These spans diffuse hemispheric and midline glioma and drivers including EGFR, PDGFRA, and ALK2, among others. We will use our novel light-activated conjugates in appropriate models to explore both efficacy and immune modulation in appropriate models, including those that may become available over the course of the project.

We have established a workflow using multi-omic technologies to characterise the tumour microenvironment in such models, dissociating tumours and profiling them using single-cell RNA-sequencing (scRNA-seq) (10x Genomics) in-house. This will be augmented by the use of spatial transcriptomics (Visium, Xenium) and imaging mass cytometry in order to place the specific immune populations within the tissue architecture. We will also leverage our 22-colour custom flow cytometry immune panel, immunohistochemistry (IHC) and immunofluorescence on tissue sections to validate these findings. Last but not least, the anti-tumour effects in response to PIT and PIT combined with i) immunotherapies and ii) potentially novel targeted agents (e.g. ONC201) will be analysed in selected *in vivo* models. After treatment tumour response will be monitored by MRI. Imaging data will be also correlated with IHC staining. In addition, brain, spleen or TD lymph nodes will be collected post-PIT at different time points for both local tumour control and immunological effects (numbers and functional activation/exhaustion state of tumour infiltrating cells by flow cytometry panels and IHC, circulating cytokines).

Literature references

1. Jones, C., et al., *Pediatric high-grade glioma: biologically and clinically in need of new thinking.* Neuro Oncol, 2016. **19**: p. 153-161.

2. Taylor, K.R., et al., *Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma.* Nat Genet, 2014. **46**(5): p. 457-61.
3. Mackay, A., et al., *Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma.* Cancer Cell, 2017. **32**(4): p. 520-537 e5.
4. Mackay, A., et al., *Molecular, Pathological, Radiological, and Immune Profiling of Non-brainstem Pediatric High-Grade Glioma from the HERBY Phase II Randomized Trial.* Cancer Cell, 2018. **33**(5): p. 829-842 e5.
5. Nussbaumer, G., et al., *HGG-49. Gliomatosis cerebri in children: A collaborative report from the European Society for Pediatric Oncology (SIOPE).* Neuro-Oncology, 2022. **24**(Supplement_1): p. i72-i73.
6. Sievers, P., et al., *A subset of pediatric-type thalamic gliomas share a distinct DNA methylation profile, H3K27me3 loss and frequent alteration of EGFR.* Neuro Oncol, 2021. **23**(1): p. 34-43.
7. Nakajima, K., et al., *Changes in plasma membrane damage inducing cell death after treatment with near-infrared photoimmunotherapy.* Cancer Sci, 2018. **109**(9): p. 2889-2896.
8. Burley, T.A., et al., *Near-infrared photoimmunotherapy targeting EGFR-Shedding new light on glioblastoma treatment.* Int J Cancer, 2018. **142**(11): p. 2363-2374.
9. Maczynska, J., et al., *Triggering anti-GBM immune response with EGFR-mediated photoimmunotherapy.* BMC Med, 2022. **20**(1): p. 16.

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 in a Biological Science

Intended learning outcomes:

- Develop an in-depth understanding of PDHGG biology and the tumour immune microenvironment.
- Acquire skills in immunocompetent mouse models and patient-derived orthotopic xenografts.
- Build expertise in photoimmunotherapy and necessary skills to develop these technologies further.
- Expand laboratory experience in a broad range of techniques including tissue culture, drug screening, multi-colour flow cytometry, immunohistochemistry, immunofluorescence and bulk and single cell sequencing technologies.
- Develop the ability to test self-driven hypotheses, critically appraise relevant literature and design experiments independently using robust methodology.
- Produce research outputs including project reports, conference abstracts, presentations at key meetings and peer-reviewed published papers.

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science