ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	The Rudy A Menon Foundation

Project details

Project title:	Modelling and targeting the effects of EGFR on diffusely infiltrating paediatric high-grade gliomas or midline and cerebral regions

Supervisory team

Primary Supervisor:	Chris Jones and Julia Cockle
Associate Supervisor(s):	Ketty Kessler
Secondary Supervisor:	Lou Chesler

Divisional affiliation

Primary Division:	Molecular Pathology
Primary Team:	Glioma
Site:	Sutton

Project background

High-grade gliomas in children and young adults represent a diverse collection of brain tumours with a poor clinical outcome, in part due to their diffuse nature of presentation. Integrated genomic and epigenomic studies over the last 12 years by ourselves and others has highlighted distinct subgroups of the disease with different ages of incidence, locations within the CNS, and unique biological drivers. Even within the now formalised ‘paediatric-type diffuse high-grade gliomas’ (PDHGG) there are groups of tumours with an even more pronounced pattern of diffuse infiltration, which presents a particular clinical challenge, with 2-year survival rates of <5%. In the cerebral hemispheres, gliomatosis cerebri (GC) is defined as a high-grade glial tumour which spreads through > 3 lobes, and has recently been considered a growth pattern rather than a distinct entity in itself. Through a pan-European collaborative study of >100 clinically annotated cases, we have identified a strong enrichment of GC for a specific epigenetically-defined subgroup driven by EGFR and BCOR mutations. In midline regions, tumours that extend through both thalami (bithalamic glioma) are strongly linked to a novel subtype of diffuse midline glioma-EGFR altered (DMG-EGFR), also enriched for EGFR alterations, here on the background of the H3K27 alterations prevalent in tumours of this location. We hypothesise that these 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. We further suggest that these tumours may be targeted by a number of potential therapeutic approaches, offering hope for clinical progress for children and young adults with these tumours for the first time.

Project aims

- Modelling of EGFR-driven GC and DMG by in utero electroporation.
- Targeting EGFR by small molecules and immunotherapies
- Modulating the immune microenvironment with oncolytic virus delivery of an EGFR-targeting T-cell engager

Research proposal

Modelling of EGFR-driven GC and DMG by in utero electroporation

We will generate novel mouse models of EGFR-driven GC and DMG using in utero electroporation (IUE), constructing PBCAG-eGFP-EGFR_A289V and PBCAG-eGFP-EGFR_ A767delinsASVD vectors. These will be combined with PX330-Trp53 and PBCAG-eGFP-H3f3a_K27M (DMG-EGFR) or PX330-BCOR, and in order to mimic the genotypes observed in the human disease. We will target both forebrain and hindbrain precursors at a range of developmental timepoints between E11.5-15.5. Plasmids are injected and electric pulses applied using 3mm Tweezertrodes using the ECM square wave electroporation system (Harvard Apparatus). Embryos are allowed to survive until birth and monitored by bioluminescence with signal-bearing animals selected for further analysis. Tumour growth is assessed by MRI (with Simon Robinson and Jessica Boult, Division of Radiology and Imaging) and IVIS within the ICR's Centre for Cancer Imaging. Sacrificed mice will undergo molecular, histological and immunohistochemical analysis supported by a trained histopathology technician and an academic neuropathology clinical fellow within the Jones lab. Models developed in this way may be serially propagated both in vitro and orthotopically in vivo, making them amenable to high-throughput screening as well as useful for rapid preclinical efficacy testing. Effects on downstream pathway signalling will be assessed by RNA-sequencing, Western blot and quantitative proteomics (mass spectrometry, ICR proteomics facility), in addition to single cell RNA-seq (10X, Visium spatial transcriptomics)) and proteomics (CyTOF, imaging mass cytometry).

Targeting EGFR by small molecules and immunotherapies

We will screen for the efficacy of EGFR-targeted agents using both IUE-derived models as described above, as well as patient-derived models of GC- (e.g. OPBG-GC-006) and DMG-EGFR (e.g. ICR-CXJ-064) subgroups, established as 2D/3D cultures in vitro, on ex vivo organotypic brain slices, and as orthotopic xenografts in vivo. For cell-based assays, we will assess the effects on cell viability in vitro (CellTiterGlo) and invasion/migration ex vivo (Zeiss LSM980, Operetta) using multiple modes of targeting the receptor. These will include small molecule inhibitors, both first generation reversible agents such as erlotinib and gefitinib, as well as newer generation irreversible inhibitors such as afatininb and osimertinib. We will further explore the use of vandetanib, given our pan-European clinical trial of the drug in combination with everolimus (ITCC-eSMART), and monoclonal antibodies such as cetuximab and panitumumab. Furthermore, we will explore immunotherapeutic agents including CAR T-cells directed against EGFR (Nick Vitanza, Seattle) and photoimmunotherapeutic approaches using small affibody proteins with a fluorescent molecule engineered to trigger cell death upon exposure to near-infrared light (EGFR-IR700, with Gabriella Kramer-Marek, ICR). We will explore the effects on downstream pathway signalling in response to EGFR targeting by RNA-sequencing, Western blot and quantitative proteomics (mass spectrometry, ICR proteomics facility). The most promising agents will be further evaluated for their effects on survival and tumour spread in vivo, with appropriate PK/PD studies and assessment of signalling response (RNA-sequencing, mass spectrometry).

Modulating the immune microenvironment with oncolytic virus delivery of an EGFR-targeting T-cell engager

Oncolytic viruses (OV) can act as both direct cytotoxic agents, as recently shown in diffuse intrinsic pontine glioma (DIPG) and powerful immune stimuli, capable of remodelling the tumour immune microenvironment (TIME), for which we have preliminary evidence in our paediatric high grade glioma models. With Julia Cockle (RMH), we aim to evaluate the in vitro effects of OV expressing an epidermal growth factor receptor (EGFR)-targeting bispecific T-cell engager (BiTE), on patient-derived models of GC- and DMG-EGFR, exploring the effects on cell viability, apoptosis, invasion, proliferation and cytokine production. OV treatment will be evaluated in vivo using our IUE-derived mouse models, with evaluation of anti-tumour effects (tumour volume by MRI, invasion (H+E stained tissue sections), apoptosis, proliferation, degrees of necrosis and vascularisation (immunohistochemistry)), alongside survival. The effect of OV treatment on the TIME will be assessed by single cell RNA-seq (10X, Visium spatial transcriptomics) and proteomics (CyTOF, imaging mass cytometry), as well as multi-colour flow cytometry, immunohistochemistry and immunofluorescence. We will also screen the use of OV-BiTE alongside the most promising EGFR-targeted therapies above, with in vitro experiments evaluating the effect of promising combinations on viral replication, apoptosis, cytokine secretion, immunometabolic signatures and cellular pathways. Promising combinations will also be investigated in vivo (alone and in combination with radiotherapy) to determine anti-tumour effects and to evaluate the effect on the TIME using our established workflow described above in our IUE-derived mouse models.

Literature references

[1] Sievers P, Sill M, Schrimpf D, …, Jones C, Witt O, Pfister SM, von Diemling A, Jones DTW and Sahm F (2021) “A subset of pediatric thalamic gliomas share a distinct methylation profile and frequent alteration of EGFR” Neuro-Oncology 23(1):34-43

[2] Mackay A, Burford A, Carvalho D, … and Jones C (2017) “Integrated molecular meta-analysis of 1000 pediatric high grade glioma and diffuse intrinsic pontine glioma” Cancer Cell {Cover} 32(4):520-537

[3] Korshunov A, Schrimpf D, Capper D, … Pfister SM and Jones DTW (2017) “H3/IDH wild-type pediatric glioblastoma is comprised of three molecularly and prognostically distinct subsets” Acta Neuropathologica 134(3):507-516

[4] Mączyńska J, Raes F, Da Pieve C, Turnock S, Boult JKR, Hoebart J, Niedbala M, Robinson SP, Harrington KJ, Kaspera W, Kramer-Marek G. (2022) “Triggering anti-GBM immune response with EGFR-mediated photoimmunotherapy.” BMC Med. 20(1):16.

[5] Broniscer A, Chamdine O, Hwang S, Lin T, Pounds S, Onar-Thomas A, Shurtleff S, Allen S, Gajjar A, Northcott P, Orr BA (2016) “Gliomatosis cerebri in children shares molecular characteristics with other pediatric gliomas. .Acta Neuropathol. 131(2):299-307.

[6] Vitanza NA, Johnson AJ, Wilson AL, …, Jensen MC, Park JR (2021) “Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis.” Nat Med. 27(9):1544-1552.

[7] Pérez-Larraya JG, Garcia-Moure M, Labiano S, …, Fueyo J, Gomez-Manzano C, Tejada S and Alonso MM (2022) “Phase 1 Trial of DNX-2401 Oncolytic Virotherapy for Newly Diagnosed Pediatric Diffuse Intrinsic Pontine Glioma” New Engl J Med 386(26):2471-2481

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 biological sciences

Intended learning outcomes:

- Paediatric glioma biology
- Single cell profiling
- Cell culture and molecular biology
- Animal handling and in utero electroporation
- Drug assays
- Immunotherapy

Advertising details

Project suitable for a student with a background in:

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