The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Evolutionary dynamics of MEK inhibitor sensitivity and resistance in diffuse midline glioma**

SUPERVISORY TEAM	
Primary Supervisor(s):	Chris Jones
Associate Supervisor(s):	Rebecca Rogers Mimi Bandopadhayay, Dana-Farber Cancer Institute, Boston
Backup Supervisor:	Olivia Rossanese
Lead contact person for the project:	Chris Jones
DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Glioma

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Diffuse midline glioma with H3K27M mutation (DMG) comprise diffuse intrinsic pontine glioma (DIPG, arising in the pons) and high grade glioma occuring in other midline regions such as the thalamus, and are defined by the presence of a recurrent lysine-to-methionine substitution on genes encoding histone H3 [1]. These occur almost exclusively in children, and have a median overall survival of 9-12 months, with the vast majority of patients succumbing to their disease within 2 years of diagnosis [2]. New treatments are clearly desperately needed, and to date no experimental therapies have shown substantive increases in survival in the clinic.

We have recently undertaken large scale molecular analyses of both retrospective cohorts and prospective clinical trial samples in DMGs and other paediatric high grade glioma [3-5]. These analyses have highlighted recurrent co-segregating mutations in distinct subgroups of DMGs in genes driving activation of the mitogen-activated protein kinase (MAPK) pathway. Specifically, non-brainstem H3.3K27M DMGs have been identified to harbour either somatic *NF1* missense or truncating mutations, hotspot kinase mutations in *FGFR*1, or alternatively internal tandem duplications in *NTRK2*. By contrast, DIPGs with H3.3K27M (but not those in genes encoding H3.1) have frequent small insertions or deletions (InDels) in *PIK3R*1, and we have also identified a case with a non-canonical *BRAF* G469V mutation. Notably, such mutations are often found to be subclonal, and analyses of pre-post tumors in the DIPG-BATs trial [3] and whole brain autopsy studies reveal extensive heterogeneity of these alterations across the diffusely infiltrating tumour, with evidence of convergent evolution.

In other tumour types with activated MAPK signalling, targeted inhibition of MEK has proven to be a useful therapeutic approach, including paediatric brain tumour patients [6, 7], and is an unexplored option for DMGs. Within our prospective co-clinical trial of UK BIOMEDE patients, *in vitro* drug screening of primary patient-derived DMG models shows that these mutations may confer a differential sensitivity to MEK inhibitors such as trametinib. Importantly however, resistance

mechanisms have been shown to limit the long-term effectiveness of these inhibitors clinically [8, 9]. In our *BRAF* G469V DIPG model, we were able to demonstrate similar caveats with single agent therapy, with the emergence of multiple *MEK1* (K57N, I143S) and *MEK2* (I115N) mutations in distinct clones from continuous *in vitro* exposure experiments. Such resistant clones are conversely differentially sensitive to dasatinib (multi-kinase PDGFR/SRC inhibitor), and combined trametinib / dasatinib was found to be synergistic in the biopsy models, and to block the emergence of resistance over continuous passaging.

PROJECT AIMS

We propose that subgroups of diffuse midline glioma with H3K27M mutation (DMG) harbour mutations which confer sensitivity to targeted MEK inhibitors, but that resistance mechanisms will limit their clinical effectiveness.

- We will determine the frequency of such alterations and characterise novel patient-derived models of such tumours prospectively via a biopsy-stratified clinical trial.
- We will use these models to test the prediction that DMGs harbor distinct populations of cells that are predetermined to exhibit tolerance to MEK inhibition through high complexity barcoding.
- Finally, we hypothesise that treatment of DMG cells with MEK-inhibitors will induce novel genetic dependencies that can be targeted in combination therapies, and we will leverage genome-scale CRISPR-Cas9 screens to identify such combinations for validation and clinical translation in this devasting disease.

RESEARCH PROPOSAL

- <u>Novel models and clinical profiling of MAPK-driven DMG</u>

  As part of the prospective BIOMEDE clinical trial, we have to-date established 20 novel patient-derived *in vitro* / *in vivo* DIPG models, including those with pathogenic MAPK alterations. As this study expands to other DMGs, we will continue to establish novel cultures *in vitro* (2D on laminin, 3D neurospheres) and PDX models *in vivo* (orthotopic implantation in immunocompromised mice), in addition to those collected routinely through our centres and collaborators. Where possible, as well as the biopsy material mandated as part of the trial, we will collect post-mortem tumour tissue from patients whose families have consented to a rapid autopsy protocol. In addition to our usual whole exome sequencing, methylation arrays profiling and bulk RNA sequencing, we will undertake single-cell RNAseq using the 10X Chromium system of 2500 single cells from per sample to a depth of 50,000 reads, allowing for detection of gene expression, transcribed fusion genes, splice variants and SNVs as well as inferred CNAs. We will further collect plasma and CSF from patients for the analysis of cell-free DNA for non-invasive molecular profiling and monitoring of treatment response. Here we will combine ultra-low pass whole-genome sequencing and deep sequencing of bespoke gene panels to identify genome-wide CNAs, SNVs/InDels, and SVs, along with droplet PCR-based approaches for the specific detection of relevant MAPK alterations during tumour evolution.

- <u>High-complexity barcoding to characterize resistant subclones</u>

  We will first test the hypothesis that DIPG subclones exhibit predetermined resistance to MEK inhibition using a novel selectable CRISPR-Cas9 based barcoding library (EvoSeq) developed by our key collaborators (Mimi Bandopadhayay, Dana-Farber Cancer Institute, Boston) that allows us to track lineages of cells through treatment with MEK inhibitors and then retrieve subclones from the pre-treatment pool that harbour resistance barcodes. We will infect appropriate DMG cells with a pooled lentiviral library encompassing 4,000,000 unique guides using a low multiplicity of infection so that individual cells are labelled with single barcodes. We will expand this population of

cells (which will allow the propagation of barcodes to daughter cells) and will then initiate treatment with candidate small molecule inhibitors in replicate experiments (while also freezing a pool of viable barcoded cells for future characterization). We will also extract DNA prior to initiation of therapy and on acquisition of resistance from each independent replicate to determine abundance of individual guide barcodes in each sample by PCR-amplification and Illumina sequencing. The number of reads for each barcode will be normalized between samples and log2-transformed. The RIGER algorithm in the GENE-E Java package will be applied to generate a ranked list of statistically significant enriched barcodes (based on a comparison of log-fold change) in the population of cells that are resistant to candidate small-molecule inhibitors relative to untreated cell lines.

The barcodes that persist after treatment with inhibitors will be compared between replicates to determine whether there is significant overlap, indicating pre-existing resistant clones. Alternatively, if resistance is stochastic, random non-overlapping barcodes will be recovered. If we detect a predetermined pattern of resistance, we will retrieve cells that harbour 'resistance' marking barcodes (from the pre-treatment pool) by flow cytometry. We will validate these cells to harbour innate resistance to a panel of MEK inhibitors (trametinib, selumetinib and cobimetinib) in low-throughput assays. We will then characterize the molecular phenotype of the cells to identify potential resistance drivers. Assays will include whole-exome sequencing to identify genetic drivers of resistance, RNA-sequencing (single-cell and bulk) and ATAC-sequencing to identify transcriptomic and epigenetic drivers of resistance.

- <u>High-throughput combinatorial screens</u>

  Treatment of cancer cells with small-molecule inhibitors lead to genetic and transcriptional changes. Our key collaborators (Mimi Bandopadhayay, Dana-Farber Cancer Institute, Boston) have found such changes to induce novel dependencies of tumor cells in the context of treatment of other inhibitors such as BET-bromodomain inhibitors [10]. Here, we will apply genome-scale modifier screens to identify genes, which when ablated, confer sensitivity to low doses (IC25 dosing) of MEK inhibitors.

  The Bandopadhayay lab have optimized methodologies to perform genome-scale CRISPR-Cas9 assays in neurosphere models of brain tumors. We will perform these screens in the presence of the MEK inhibitor trametinib (or vehicle control) to identify genes, which when suppressed, increase the efficacy of the inhibitor. They have access to a pooled library of 73,687 unique guide sequences (sgRNA) that target 18,454 genes (Avana library), each associated with a unique barcode. This library utilizes a single lentiviral vector to deliver Cas9, a sgRNA and an antibiotic selection marker. Two independent DMG stem cell lines will be infected with the pooled lentiviral library, with a target infection efficiency of 30-50% to minimize the chance of multiple vector integrations per cell. Cells will be passaged for three weeks after transduction and puromycin selection in the presence of trametinib (IC25 dosing) or vehicle control. DNA will be extracted at baseline and after propagation for three weeks. During passaging, a minimum representation of 1000 cells per clone will be maintained. The relative abundance of each sgRNA will be determined at the end of the assay relative to baseline, and sgRNAs that are depleted at the last time point represent genes that synergize with trametinib in DIPG cells.

  We will validate candidate hits in low-throughput assays, leveraging CRISPR-Cas9 and short-hairpin assays to determine effects on cell-proliferation, cell-cycle and apoptosis, expanding the panel of MEK inhibitors and DMG cell-lines assessed. For those dependencies for which small molecule inhibitors exist, we will assess whether these compounds exhibit synergy with MEK inhibitors, using assays where we measure ATP content as a marker of proliferation [10]

LITERATURE REFERENCES

1. Jones, C. and S.J. Baker, *Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma.* Nat Rev Cancer, 2014. **14**(10).
2. Jones, C., et al., *Pediatric high-grade glioma: biologically and clinically in need of new thinking.* Neuro Oncol, 2016.
3. Gupta, N., et al., *Prospective feasibility and safety assessment of surgical biopsy for patients with newly diagnosed diffuse intrinsic pontine glioma.* Neuro Oncol, 2018. **20**(11): p. 1547-1555.
4. 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.
5. 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.
6. Banerjee, A., et al., *A phase I trial of the MEK inhibitor selumetinib (AZD6244) in pediatric patients with recurrent or refractory low-grade glioma: a Pediatric Brain Tumor Consortium (PBTC) study.* Neuro Oncol, 2017. **19**(8): p. 1135-1144.
7. Kondyli, M., et al., *Trametinib for progressive pediatric low-grade gliomas.* J Neurooncol, 2018. **140**(2): p. 435-444.
8. Gao, Y., et al., *Allele-Specific Mechanisms of Activation of MEK1 Mutants Determine Their Properties.* Cancer Discov, 2018. **8**(5): p. 648-661.
9. Mandal, R., S. Becker, and K. Strebhardt, *Stamping out RAF and MEK1/2 to inhibit the ERK1/2 pathway: an emerging threat to anticancer therapy.* Oncogene, 2016. **35**(20): p. 2547-61.
10. Bandopadhayay, P., et al., *Neuronal differentiation and cell-cycle programs mediate response to BET-bromodomain inhibition in MYC-driven medulloblastoma.* Nat Commun, 2019. **10**(1): p. 2400.

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 a relevant biological science
Intended learning outcomes:	• Ability to carry out cutting-edge high-throughput screening techniques and single cell profiling • An understanding of resistance mechanisms and combination therapies in the targeted treatment of cancer • Knowledge of the underlying biology of childhood diffuse midline glioma • Skills in critical thinking, experimental design and interpretation • Training in appropriate bioinformatic analyses
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. DIPG / midline glioma**
	2. Childhood brain tumours
	3. MAPK
	4. Resistance
	5. CRISPR screening
	6. Cancer evolution