The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Regenerating the genetic predisposition to medulloblastoma using human neuroepithelial stem cells**
Short Project Title:	***Regenerating medulloblastoma predisposition using genetically manipulated human stem cells***

SUPERVISORY TEAM	
Primary Supervisor:	Louis Chesler
Other members of the supervisory team:	Jignesh Tailor and Rajesh Chopra

DIVISIONAL AFFILIATION	
Primary Division:	Clinical Studies
Primary Team:	Paediatric Solid Tumour Biology and Therapeutics
Other Division:	Cancer Therapeutics

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Medulloblastoma (MB) is the most common malignant brain tumour in children. Four molecular subgroups have been identified through large-scale genomic studies, and approximately one-third of patients have the sonic hedgehog (SHH) subtype. Mutations in the SHH-receptor Patched (PTCH) are found in 25% of this sub-type. A significant proportion of SHH and group 4 tumours also harbor aberrant N-MYC signaling. Mouse models have provided critical insight into mechanisms of MB initiation. However, they are difficult to construct and the experimental tumours do not fully recapitulate the mutational complexity of human disease. The co-operative events that initiate transformation of premalignant cells to MB in humans is still largely unknown.

Recent advances in stem cell technology have led to novel *in vitro* culture systems that permit maintenance and high-throughput genetic manipulation of hindbrain precursors using CRISPR/Cas9 and SMaSH tagging. Stable, long-term neuro-epithelial stem (NES) cells with hindbrain identity and ability to form cerebellar cells have been generated from human induced pluripotent stem (iPS) cells and directly from human hindbrain tissue. These cells represent a candidate cell-of-origin for human MB, and recent studies show that NES cells harbouring germ-line mutations in PTCH or iPS-derived NES transduced with N-MYC generate SHH-subtype medulloblastoma tumours in vivo.

The goal for this PhD studentship is to use these existing methods and advanced gene-manipulation to generate implanted models of medulloblastoma from human fetal hindbrain derived NES cells, which have been gene-edited to over-express N-MYC. NES cells driven by N-MYC will provide the unique platform to discover novel N-MYC pathway-targeted therapeutic agents through existing drug screening libraries in-place at the ICR. The project will uncover mechanisms and therapeutic approaches useful to combat this fatal disease in children, and will provide a unique opportunity in therapeutic drug screens and target identification, both core strengths of the ICR.

PROJECT AIMS

Aim 1: To generate SHH-subgroup medulloblastoma initiating cell lines by transforming N-MYC into foetal hindbrain derived neuroepithelial stem (NES) cells using existing methods and to generate neuroepithelial stem cell cultures from medulloblastoma tissue obtained from orthotopic mutant-NES cell transplants and patients.

The student will learn cutting edge technology in stem cell culture, and essential skills in cancer stem cell biology, including PCR, immunostaining, genetic manipulation of cells with CRISPR technology, and orthotopic transplantation of stem cells into mice

Aim 2: To identify N-MYC pathway-targeted compounds that inhibit the growth of medulloblastoma initiating cells using existing medicinal chemistry drug screening libraries available within the ICR drug development unit, and to demonstrate the activity of selected drugs in vitro and in mice orthotopically transplanted with medulloblastomas derived from mutant NES cells

The student will learn how to design and execute a stem cell based drug screen and to identify therapeutic pathways that may inhibit growth of tumour stem cells, and design in vivo therapeutic experiments in mice

RESEARCH PROPOSAL

Human foetal hindbrain derived neuroepithelial stem (NES) cells are long term propagating human cells in culture that maintain hindbrain regional identity, and potency for human cerebellar cells both *in vitro* and *in vivo* (Tailor et al., 2013). In mouse, genetic perturbations in Math1-expressing granule neuron precursors is sufficient to generate medulloblastoma (Yang et al., 2008; Schuller et al., 2008), and these models have provided great insight into the mechanisms and development of this disease. Since human hindbrain NES cells maintain potency for GNP-like precursors, they represent candidate cells of origin for human medulloblastoma. Moreover, they can be modelled from embryonic stem (ES) cells derived from the human embryo, or induced pluripotent stem (iPS) cells derived from patients with genetic predisposition for medulloblastoma such as Gorlin syndrome (Huang & Tailor et al., *Cell Stem Cell*, in review).

Recent experiments have shown that over-expression of N-MYC in normal iPS cell-derived NES cells can generate SHH-subgroup medulloblastoma *in vivo*, which are more reminiscent of human medulloblastoma than their mouse counterparts (Huang & Tailor et al., Cell Stem Cell, in review). The first aim of this PhD is to generate human medulloblastoma *in vitro* and *in vivo* models through transduction of N-MYC overexpression in hindbrain neuroepithelial stem cells derived directly from the human foetal brain (Tailor et al., 2013). Our PhD student will learn how to maintain human neuroepithelial stem cells in culture,

transduce and gene-tag these cells with existing CRISPR-Cas9 gene-editing tools (Bressan et al., 2017). Cells stably transduced with N-MYC (and other candidate MB driver genes) will be characterised *in vitro* and subsequently transplanted into mouse brain to generate xenograft models of medulloblastoma.

In parallel, the student will collect medulloblastoma tissue from these xenograft models, and patients with medulloblastoma (through existing collaborations in place with our partner hospitals), with the aim to re-derive cancer stem cell lines that are similar to NES cells using established culture techniques (Tailor et al., 2013, Pollard et al., 2009). The expected outcome is to derive NES cell lines or human medulloblastoma derived cell lines that reliably generate medulloblastoma after orthotopic transplantation into mice. We will analyse the xenograft tumours derived from the NES cells against existing mouse models and human medulloblastoma to determine how representative these tumours are for the human disease using genetic profiling tools.

N-MYC pathway genes are overexpressed in human SHH-subgroup and Group 4 medulloblastoma, suggesting that it plays a vital role in the development and maintenance of this disease. The group 4 subgroup, in particular, represent a very malignant variant of medulloblastoma, and is highly understudied (Kool et al., 2012). Therapeutic targets for both SHH and group 4 medulloblastoma are lacking. Once the human medulloblastoma xenograft model from hindbrain derived NES cells is established, the student will be in a position to screen the medulloblastoma-intiating NES cells with established N-MYC target drug libraries available at the ICR. This part of the project will be performed in collaboration with Raj Chopra's lab within the ICR drug development unit.

One of the drug screening libraries developed by Chopra's team exploits a novel protein degradation mechanism involving the E3 ubiquitin ligase. Following small molecule modulation of thalidomide derivatives E3 ubiquitin ligases can acquire neo-substrates. A novel proprietary CUL4 ^{CRBN} E-3 ligase chemical library has been developed by Chopra's team and will be used on N-MYC driven medulloblastoma-derived stem cells to underpin the mechanisms of N-MYC dependency, and to identify novel therapeutic agents that may tackle N-MYC dependent mechanisms in these cells. Therapeutic agents that limit the growth of these cells in culture will be selected for further testing both with *in vitro* assays, and also in mice harbouring orthotopic xenograft models of medulloblastoma. The expected outcomes will be the identification of novel therapeutic agents that act against key proteins involved in N-MYC dependency to curtail the growth of medulloblastoma.

LITERATURE REFERENCES

Bressan RB, Dewari PS, Kalantzaki M, Gongoso E, Matjusaitis M, Garcia-Diaz C, Blin C, Grant V, Bulstrode H, Gogolok S, Skarnes WC, Pollard SM. Efficient CRISPR/Cas9-assisted gene targeting enables rapid and precise genetic manipulation of mammalian neural stem cells. Development 144(4): 635-648 (2017).

Huang M, Tailor et al., Recapitulation of genetic predisposition to medulloblastoma in human neuroepithelial stem cells. Cell Stem Cell (2018) (in review)

Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, Cho YJ, Koster J, Schouten-van Meeteren A, van Vuurden D, Clifford SC, Pietsch T, von Bueren AO, Rutkowski S, McCabe M, Collins VP, Bäcklund ML, Haberler C, Bourdeaut F, Delattre O, Doz F, Ellison DW, Gilbertson RJ, Pomeroy SL, Taylor MD, Lichter P, Pfister SM. Molecular subgroups of medulloblastoma: an international meta-

analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. Acta Neuropathol. 2012 Apr;123(4):473-84
Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, Squire JA, Smith A, Dirks P. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell. Jun 5;4(6):568-80 (2009).
Schüller U, Heine VM, Mao J, Kho AT, Dillon AK, Han YG, Huillard E, Sun T, Ligon AH, Qian Y, Ma Q, Alvarez-Buylla A, McMahon AP, Rowitch DH, Ligon KL. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. Cancer Cell.14(2):123-34 (2008)

Tailor J, Kittappa R, Leto K, Gates M, Borel M, Paulsen O, Spitzer S, Karadottir RT, Rossi F, Falk A, Smith A. Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency. J Neurosci 33(30): 12407-22 (2013)

Yang ZJ, Ellis T, Markant SL, Read TA, Kessler JD, Bourboulas M, Schüller U, Machold R, Fishell G, Rowitch DH, Wainwright BJ, Wechsler-Reya RJ. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. Cancer Cell. 14(2):135-45 (2008)

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 in biochemistry, neuroscience, stem cell biology, genetics or cancer biology
Intended learning outcomes:	• Cutting edge technology in neural stem cell culture • How to genetically manipulate stem cells in culture with CRISPR-Cas9 gene editing tools • Orthotopic transplantation of human stem cells into mouse brain for disease modelling • Design and execution of a drug screen in human neural stem cells • Novel approaches to drug discovery • Develop an understanding of synthetic lethality in cancer that would translate into novel therapeutic targets in medulloblastoma