The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	The Institute of Cancer Research (ICR)
PROJECT DETAILS	
Project Title:	**Single-cell transcriptomics linked to lineage tracing to interrogate the role of "*plasticity first*" as a positive force driving paediatric cancer evolution**
SUPERVISORY TEAM	
Primary Supervisor:	Dr Alejandra Bruna
Associate Supervisor:	Dr Marco Bezzi Professor Louis Chesler
Secondary Supervisor:	Professor Chris Jones
DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	The Preclinical Modelling of Paediatric Cancer Evolution team
Other Division (if applicable):	Centre for Paediatric Oncology Experimental Medicine| Centre for Cancer Evolution

SHORT ABSTRACT

Darwinian evolution is inherently deficient in explaining evolution-Evolutionary theories of phenotypic plasticity as drivers of evolutionary change have been discussed for over a century

Cell plasticity (eg:EMT) is **fundamental in development** and has recently been observed as a response to therapeutic pressure and immune evasion

Peadiatric cancers are developmentally imprinted and most show transcriptional heterogeneity reminiscent of developmental stages

We hypothesize Cell plasticity/Phenotypic switching is a positive cancer evolutionary force- which is especially relevant in children's cancer (developmentally imprinted) and may allow prediction of treatment resistance

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BACKGROUND TO THE PROJECT

Children's cancers are developmentally imprinted, and cell phenotypic switches or cell plasticity is a fundamental mechanism that governs development. We hypothesize cell plasticity/phenotypic switching is a positive cancer evolutionary force which is especially relevant in children's cancer and may allow prediction of treatment resistance.

The intrinsically heterogeneous nature of cancer engages evolutionary forces in response to treatment stress. Intra-tumour heterogeneity (ITH) is shaped by/interplays with cell population dynamics, cell-extrinsic and intrinsic selective forces, and cellular adaptations. How ITH influences evolutionary paths and vice-versa remains central to understand how to effectively treat cancer. Recently, evidence indicated a major role of non-genetic mechanisms in evolution such as cell plasticity and epigenetic rewiring as drivers of cancer progression and therapeutic resistance. We hypothesise that non-genetic evolutionary processes are particularly relevant in paediatric cancers and where developmental imprinting restricts evolutionary mechanisms to those used during normal development, such as cell plasticity.

Through this proposal, we bring together expertise and resources to interrogate improved patient-derived preclinical models and cell lines with single cell technologies. This will generate a comprehensive atlas of one of the most common and most aggressive solid paediatric malignancies, medulloblastoma§, to develop innovative and rationalised therapeutic strategies for high-risk disease. We aim to; 1/ map molecular alterations in medulloblastoma that influence evolutionary paths/treatment responses and determine whether cell plasticity is a major driver of adaptive evolution and disease progression. 2/ trace single cells and state/ fate decisions under therapy stress ex vivo. We will use single cell barcoded cell cultures to identify the molecular mechanisms activated during the onset of therapy resistance in medulloblastoma. This will be achieved using cutting-edge single cell-barcode linked with single cell (sc)RNA-seq technologies.

By identifying the molecular events underpinning medulloblastoma evolutionary dynamics, we could open up the possibility to advance innovative and mechanistically-based targeted combination therapies. This study is key to evaluate whether we should collectively turn our efforts towards identifying specific innovative treatments that overcome drug resistance based on disabling the molecular mechanisms regulating adaptation.

PROJECT AIMS

Aim 1. To map in detail molecular changes in medulloblastoma that drives the development of resistance.

We will study the impact on therapeutic responses and evolutionary paths of clonal dynamics in cell lines and patient derived ex vivo models of this malignancy using cutting-edge single cell-barcode together with scRNA-seq in treated with standard of care chemotherapeutic drugs and in a flexible ex vivo system we have developed. We will expand the capabilities of this ex vivo system to explore the role of "plasticity first" as a positive driver of evolution by adapting Waddington's experiments of genetic assimilation. Barcoded cells will be serially re-plated into the treatment naïve environment and increasing doses of drugs used in frontline treatment until resistance is achieved. We will isolate cells with the resistant phenotype at different rounds of treatment and test if and when these dominant cell traits become hereditable. This framework will help identify evolutionary principles driving the plasticity of cancer cells and how these impacts on resistance to treatment and disease progression.

Aim 2. To identify early cell state/phenotype predictive of future cell fates/behaviours.

We will leverage the lineage tracing capability embedded in our system to identify pre-existing non-genetic signatures predictive of future cell identity (dominant). We will trace these back to the original baseline pools (treatment naïve cell populations) barcodes commonly found in post-treated cell pools (resistant population). By linking cellular barcodes to transcriptional information, we will extract/explore molecular signatures putative of predicting later dominance. We will also adopt a recently developed methodology, Rewind, that combines genetic barcoding with RNA fluorescence in situ hybridisation to directly capture and study cells that give rise to future cell identities of dominance/resistance. Together this will identify the molecular traits in the early stages of medulloblastoma evolution that drive clonal dominance which can be translated into improved therapeutic strategies based on early/upfront drug combinations to prevent recurrence.

Aim3. We will identify innovative treatment strategies based on evolutionary knowledge highlighted from aims 1-2 that target the emergence of resistant clones in medulloblastoma. We will evaluate promising strategies in genetically-defined, PDXs and implanted organoid systems to develop robust data on the efficacy of approaches.

RESEARCH PROPOSAL

Medulloblastoma, the most common malignant childhood brain tumour, is a major area of clinical unmet need, since relapse is common and survival following relapse is rare. Moreover, conventional standard-of-care approaches to treatment require combinations of radiotherapy and chemotherapy following initial surgery, and are associated with severe neurocognitive and endocrine late effects, especially when given to younger children. MYC-amplification characterises MBGroup3 and this amplified tumour group carries the most devastating prognoses in the disease (<10% survival).

The purpose of our research is to build a comprehensive temporal evolutionary picture, at single cell resolution, to define how medulloblastoma evolves during the formation of tumours and in response to both chemo- and targeted therapy.

Significantly, paediatric tumours have dependencies on developmentally regulated pathways. During development, genetically identical cell/clones switch between different cell phenotypes/states (cell plasticity). We hypothesise that non-genetic forces are particularly relevant in paediatric cancer where tumour cells retain cell plasticity that directs adaptive evolution. Identifying the molecular mechanisms shaping evolution may present a formula for therapeutic opportunities which could be turned into superior treatment responses and a means to prediction. This highlights a potential by which single-cell-omics and lineage tracing experiments could be used to identify targetable mechanisms driving evolution and adaptation in progressing medulloblastoma.

In this proposal, we aim to use state-of-the-art technologies to track in the fourth dimension (time) the evolution of defined cell populations using lentiviral delivery-based barcode strategies with a single cell read-out coupled with single cell transcriptomic (scRNA-seq) profiling in preclinical models of medulloblastoma..

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LITERATURE REFERENCES

1 Nowell PC. The clonal evolution of tumor cell populations. Science (80-) 1976; 194: 23 LP – 28.

2 Martincorena I, Raine KM, Gerstung M, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. Cell 2017; 171: 1029-1041.e21.

3 Turajlic S, Sottoriva A, Graham T, Swanton C. Resolving genetic heterogeneity in cancer. Nat Rev Genet 2019; 20: 404–16.

4 Gröbner SN, Worst BC, Weischenfeldt J, et al. The landscape of genomic alterations across childhood cancers. Nature 2018; 555: 321.

5 Morrissy AS, Garzia L, Shih DJH, et al. Divergent clonal selection dominates medulloblastoma at recurrence. Nature 2016; 529: 351–7.

6 Schramm A, Köster J, Assenov Y, et al. Mutational dynamics between primary and relapse neuroblastomas. Nat Genet 2015; 47: 872–7.

7 Karlsson J, Valind A, Holmquist Mengelbier L, et al. Four evolutionary trajectories underlie genetic intratumoral variation in childhood cancer. Nat Genet 2018; 50: 944–50.

8 Andersson N, Bakker B, Karlsson J, et al. Extensive clonal branching shapes the evolutionary history of high-risk pediatric cancers. Cancer Res 2020; : canres.3468.2019.

9 Luria, S. E.; Delbrück M. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. Genetics 1943; 28: 91–511.

10 Sharma S V, Lee DY, Li B, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010; 141: 69–80.

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 Biology, Biochemistry or similar.
Intended learning outcomes:	• To become proficient on single cell RNA-sequencing approaches and analysis • To develop and validate patient derived tumour cell cultures for functional genomics experimentation • To develop molecular biology techniques to unravel the biological mechanisms of transcriptional regulation and plasticity in cancer evolutionary processes

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. Single cell RNA-sequencing
	2. Lineage tracing
	3. Cell plasticity
	4. Therapy Resistance
	5. Paediatric solid cancer
	6. Cancer evolution