ICR The Institute of Cancer Research

UKRI Medical Research Council

PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Targeting MYCN via drugs that recapitulate ATRX Loss-of-Function

Supervisory team

Primary Supervisor:	Prof. Louis Chesler
Associate Supervisor(s):	Dr Evon Poon
Secondary Supervisor:	Dr Paul Clarke

Divisional affiliation

Primary Division:	Clinical Studies
Primary Team:	Paediatric Oncology Experimental Medicine
Site:	Sutton

Project background

Neuroblastoma (NB) is a common solid tumour of children with variable clinical outcome, ranging from spontaneously regressing disease in infants, to aggressive chemorefractory and widely metastatic tumours in older children(1,2). These children represent a cohort with highest unmet need for whom few suitable treatments exist to alter a dismal 3-year overall survival rate of ~15%(1,2). The genomic changes that define aggressive neuroblastoma are incompletely known, but 40% of high-risk tumours harbour amplification of the proto-oncogene MYCN(1,3,4). MYCN is a transcription factor implicated in development of neuroblastoma and other poor outcome paediatric tumours including glioblastoma and medulloblastoma. Genetic knockdown of MYCN blocks initiation of these cancers in multiple mouse models and causes nearly universal destruction of established tumours in vivo(5,6), highlighting the critical importance of MYCN as a therapeutic target. ATRX (Alpha Thalassemia/Mental Retardation, X-linked) is a tumour suppressor that is frequently mutated in paediatric tumours. ATRX regulates transcriptional stress through its role as modifier of histone methylation, with activity specifically enriched within regions of actively transcribing DNA.

In NB, missense and nonsense mutations within the 5′ coding region of ATRX co-segregate with aberrant expression of an in-frame fusion (IFF) protein which exhibits aberrant interaction with chromatin, an inability to ensure fidelity of heterochromatin structure and improper maintenance of transcriptional control mechanisms. Loss-of-function mutations in ATRX, however never segregate with MYCN gain in patients, and targeted KO of ATRX in MYCN amplified cells and mouse models is lethal, coinciding with aberrant stabilisation of R-loops within actively transcribed promotors. Taken together, these data constitute

exceptionally strong evidence that the synthetic lethal interaction between MYCN-gain and ATRX loss has a mechanistic basis that can be exploited for therapeutic benefit. This project will probe the basis for the synthetic lethality in order to identify small-molecule compounds that could selectively target MYCN-driven neuroblastoma.

Project aims

- Construct cell lines isogenic for regulatable and degradable expression of ATRX domain mutants.
- Identify specific ATRX domains with functions essential to survival of MYCN-amplified cells.
- Preclinical testing of novel and promising therapeutics.

Research proposal

The project will proceed through focusing on development of a therapeutic that recapitulates the lethality observed in MYCN driven cells upon induced loss of ATRX function.

The student will first identify the critical domain(s) of ATRX responsible for lethality in MYCN-amplified cells. Regulatable ATRX alleles will be constructed using a protein degradation-based approach using Cereblon-engaging degrader tags. The endogenous ATRX gene will be replaced with an $FKBP12^{V36F}$-tagged variant of ATRX in NB cells in a panel of MYCN-amplified cell lines. Treatment with an $FKBP12^{V36F}$-selective hetero-bifunctional small molecule will result in rapid degradation of the tagged-ATRX through recruitment of an E3-ligase that targets the fusion protein to the proteosome(7). The ATRX-dTAG models will be powerful tools by which the specific molecular and cellular impact of ATRX degradation can be probed. Genetic rescue experiments will be used to determine whether expression of WT ATRX or different domain-mutants can rescue the loss of dTAG-ATRX in an MYCN-amplified cell background. Effect on cell proliferation and viability will be evaluated in an isogenic panel of MYCN-amplified and MYCN diploid cells, using the IncuCyte live cell imaging system.

Using our existing RNA-Seq data from *MYCN*-amplified and ATRX IFF NB patients, the student will also perform genetic knockdown experiments (shRNA or CRISPR engineering) to evaluate whether the downregulation of critical proteins is synthetically lethal with MYCN amplification or ATRX alteration. The effect of these protein perturbation and on tumorigenesis in in-vivo MYCN-amplified and ATRX-mutated xenograft models will be investigated. In cases where existing small-molecule inhibitors are available focused evaluation of 2-3 compounds will be conducted in cell lines, and then in tumour xenografts and Th-MYCN GEM models of NB. This data will be required for future chemical derivatisation of candidate small-molecule drugs to take forward into lead-optimisation and clinical development.

Literature references

1. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, *et al.* Neuroblastoma. Nat Rev Dis Primers **2016**;2:16078
2. Qiu B, Matthay KK. Advancing therapy for neuroblastoma. Nat Rev Clin Oncol **2022**;19:515-33
3. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science **1984**;224:1121-4

4. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, *et al.* Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med **1985**;313:1111-6
5. Burkhart CA, Cheng AJ, Madafiglio J, Kavallaris M, Mili M, Marshall GM, *et al.* Effects of MYCN antisense oligonucleotide administration on tumorigenesis in a murine model of neuroblastoma. J Natl Cancer Inst **2003**;95:1394-403
6. Kang JH, Rychahou PG, Ishola TA, Qiao J, Evers BM, Chung DH. MYCN silencing induces differentiation and apoptosis in human neuroblastoma cells. Biochem Biophys Res Commun **2006**;351:192-7
7. Nabet B, Roberts JM, Buckley DL, Paulk J, Dastjerdi S, Yang A, *et al.* The dTAG system for immediate and target-specific protein degradation. Nat Chem Biol **2018**;14:431-41

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 (First or 2:1)

Intended learning outcomes:

- Practical knowledge and experience of wide-ranging molecular and cell biology techniques.
- Critical scientific thinking: The ability to generate and test specific hypotheses.
- Specific expertise on neuroblastoma tumour biology and MYCN transcriptional pathway.
- To obtain a Home Office Animal Licence and be able to design and implement of *in-vivo* studies.
- Scientific writing expertise for development of both thesis and publications anticipated to directly lead from this project.

Advertising details

Project suitable for a student with a background in:

- X Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science