PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	**LMO1 intracellular antibody fragments for delivery in improved treatment of childhood neuroblastoma**

Supervisory team

Primary Supervisor:	Prof. Terry Rabbits
Associate Supervisor(s):	Dr.Nikki Sereesongsaeng Dr Evon Poon
Secondary Supervisor:	Professor Louis Chesler

Divisional affiliation

Primary Division:	Cancer Therapeutics Division
Primary Team:	CTIAT
Site:	Sutton

Project background

Neuroblastoma is the most common non-brain solid tumour of children, and accounts for ~15% of childhood cancer deaths due to recurrence of metastatic, treatment resistant disease. Long term neuroblastoma survival is below 30% and classical treatment is harsh often involving high dose chemotherapy. Amplification of the oncogene MYCN is the major clinical genomic alteration associated with aggressive neuroblastoma and co-segregates with polymorphisms in LMO1, a LIM-domain only family protein (comprising LMO1, 2, 3 and 4; see https://www.icr.ac.uk/the-lmo-gene-and-protein-family). MYCN and LMO1 are key transcription factors (TFs) that cooperate to drive the core-regulatory neural plasticity circuit (CRC) which underlies clinically aggressive disease. TFs in general, and MYCN and LMO1, specifically, are proteins with intrinsically disordered domains (IDD) that are considered undruggable using traditional small-molecule chemistry. However, we successfully made LMO2 Antibody-derived binding compounds (Abd) by screening chemical libraries composed of intracellular domain antibodies capable of binding to the IDD of key TFs.

This project will use the same approach to making anti-LMO1 intracellular antibodies (iDAbs) and in turn LMO1 binding compounds. The application of these iDAbs and Abd compounds will be evaluated using isogenic cell and animal model systems that express MYCN and LMO1, and which generate aggressive noradrenergic neuroblastoma in vivo. The ability of LMO1 IDAbs to block the oncogenic activity of MYCN in these systems will be compared to that of PROTACs and iMiD-mediated molecular glues targeting LMO1 and MYCN itself, and to inducible protein-level degradation of both proteins.

The LMO1 binders generated in the project will be leads for further understanding the role of LMO1 in neuroblastoma but, most important, as lead for drug development to provide a target-specific drug that can be applied to treatment of neuroblastoma patients.

Project aims

- Library screen for anti-LMO1 intracellular domain antibody (iDAb)
- Small molecule compound screen using iDAb competition
- Catalogue SAR for hit matter expansion
- Cell-based assays to establish efficacy of iDAbs in MYCN-dependent neuroblastoma cell lines and mouse models
- Cell biology and biochemistry of LMO1 Abd lead compounds

Research proposal

Background

Neuroblastoma is a cancer that develops from neural crest cells, most often located in the adrenal gland and affecting children usually before 5 years of age. Molecular biology studies of aberrant transcription factors in the formation of neuroblastoma have shown that MYCN is a key oncogenic protein that is often over produced due to *MYCN* gene amplification. An important recent development in neuroblastoma biology is the observation that the protein LMO1 synergizes with MYCN to accelerate tumour appearance, penetrance, and metastasis [1, 2]. This synergy is a potential target for therapeutic intervention in neuroblastoma and is the subject of this proposed project.

My laboratory discovered LMO1 originally by cloning of a chromosomal translocation breakpoint in T cell acute leukaemia (T-ALL) [3] and showed that aberrant LMO1 expression in mouse T cells promoted T cell leukaemias [4]. We cloned and characterised a family of gene related to LMO1 and showed these are LIM-domain-only proteins [3], which are transcription regulators by mechanisms of protein-protein interaction (PPI) [5]. The LMO family proteins are intrinsically disordered and each one comprises two LIM domains, each with two LIM fingers. Using the family member LMO2 as an exemplar, we developed a drug discovery platform that starts with intracellular antibody fragments as molecule tools [6] and uses the intracellular antibody binding site (the paratope) to select compounds that are surrogates of the intracellular antibody. This technology was called **A**nti**b**ody-**d**erived technology (**Abd** technology) and first applied to the “undruggable” RAS protein [7] and then LMO2 [8].

The **Abd** method is applicable to any intrinsically disordered protein. In the proposed project, our work with LMO2 will be emulated to develop first LMO1-specific intracellular antibody fragments and second to implement an anti-LMO1 intracellular antibody for Abd technology application to find LMO1 drug leads. The intracellular antibody fragments will be used as tools to study LMO1 in neuroblastoma while the development of lead compound surrogates would fall outside the scope of this proposal and would be outsourced.

First generation LMO1 inhibitors: iDAb selection and characterisation

Intracellular antibody fragments binding to recombinant LMO1 will be selected using the approach outlined in figure 1. LMO1 protein will be made in *E coli* and used to screen a phage antibody library (fig 1, A) followed by candidate analysis in yeast reporter assays (B) and mammalian cells (C). Our intracellular antibody fragments are called iDAbs (for intracellular domain antibodies) and we will select those that can interfere with LMO1 protein-protein interactions in cell assays (D).

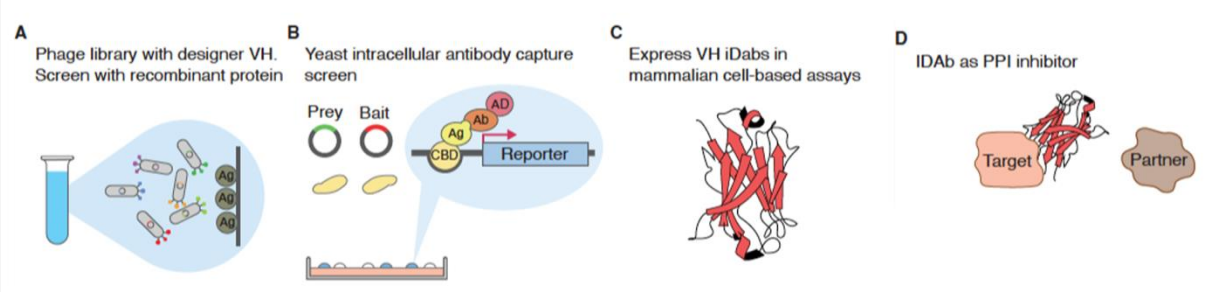

Figure 1

The activity of anti-LMO1 iDAbs will be assayed in neural crest-derived cell lines that are stably transfected with constitutive or Cre-inducible constructs that express high-levels of MYCN and/or LMO1 and enable protein level degradation with iMiDs that engage the E3 ligase cereblon. We will confirm binding of candidate iDAbs to LMO1 intracellularly using proximity ligation assays (PLA), and measure the ability of iDAbs to block the co-transcriptional stimulation of ASCL1 (through binding to the LMO1 promotor, using ddPCR of ASCL1 expression and measurement of ASCL1 promotor linked to luciferase expression). Finally, we will measure the ability of iDAbs to block expression of ADRN core-regulatory circuit genes, using ChIPSeq, RNASeq and/or digital PCR targeting ADRN CRC genes, as this is the mechanistic signature characteristic of oncogenic synergy between LMO1 and MYCN co-expression. Finally, levels of MYCN protein and engagement of MYCN target genes will be measured as an orthogonal assessment of MYCN oncogenic activity. In parallel studies, we will assess in vivo activity of lead-candidate iDAb using established MYCN-driven transgenic models of neuroblastoma, and our luciferase-tagged, fluorescent marker expressing neural crest allograft models (fig. 2, B).

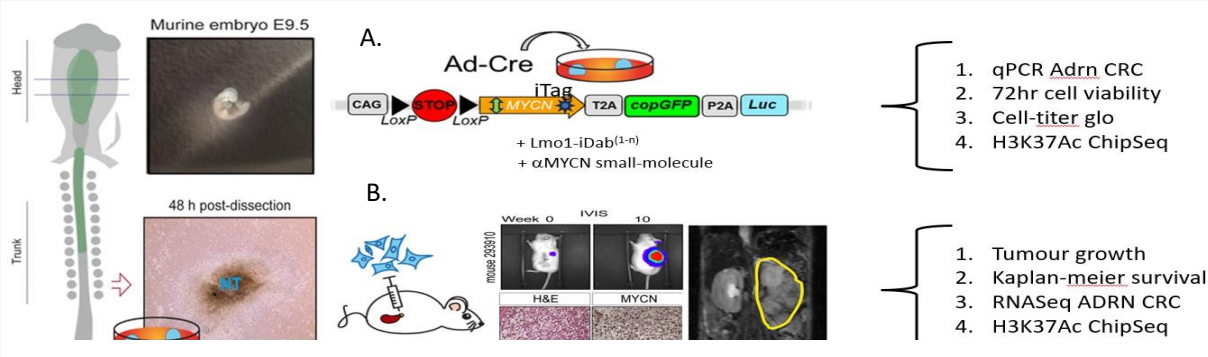

Figure 2

LMO1 compound screen using Abd technology

The student will use one selected anti-LMO1 iDAb to establish a cell-based assay with the signal generated from LMO1-iDAb interaction (as we did for LMO2-iDAb [8]). Our iDAbs correspond to the variable region fragments from whole antibody (fig 3, A, B) will be used to select paratope surrogates (C-D) from an approximately 10,000 compound triaged ICR cell-penetrating chemical library.

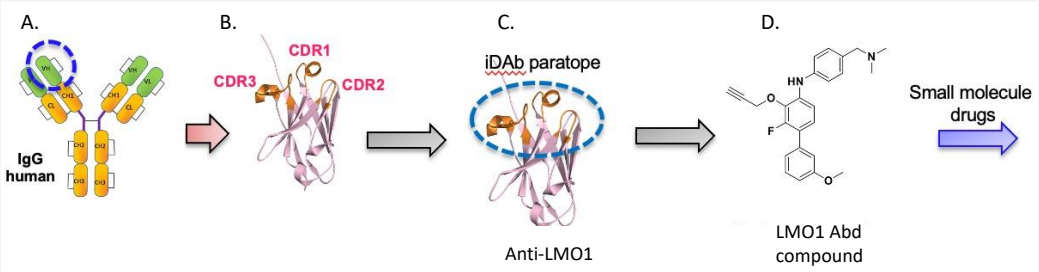

Figure 3

Hit chemical matter that is derived from the library will be confirmed by the original assay (e.g. cell-based BRET) and by an orthogonal assay, such as NMR waterLOGSY. Confirmed hits will be expanded initially using chemicals that are available commercially, to build some preliminary structure activity relationship data (catalogue SAR). Since the ICR library has been triaged for some properties that have good cell properties, and since the Abd library screen will be conducted in cells, the chemical hits will be tested in cell assays in which LMO1 function can be determined, such as PPI between LMO1 and TAL1 or LMO1 and LDB1 are two parameters.

The objective is to confirm LMO1 in cell binding by the Abd compounds and to establish some initial SAR for the full scale drug development programme. Limited medicinal chemistry will be possible with our collaborators in the Oxford LMO chemistry group.

Conclusion

LMO1 is a paralogue of LMO2 and the technologies for selecting LMO1 iDAbs, and using these for small molecule screens for paratope surrogates, are established from our own published work. Because the technologies were developed in-house, there is a high probability of success. We would expect the student to be able to achieve the isolation of chemical hit matter from an Abd screen and initial hit characterisation. The hit to lead development and lead optimisation depends very much on the chemical profile and potency. This will ultimately be outsourced.

WORK PLAN

YEAR 1

i) Antibody library screen for anti-LMO1 intracellular domain antibody (iDAb)
ii) Characterise LMO1-iDAb interaction

YEAR 2

iii) Establish cell-based BRET screening cell line
iv) Small molecule compound screen

YEAR 3-4

v) Catalogue SAR for hit matter expansion
vi) Cell-based efficacy assays

YEAR 4

vii) SAR in collaboration with Oxford LMO chemistry group
viii) Cell biology and biochemistry of lead compounds

Literature references

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8. Bery, N., C.J.R. Bataille, A. Russell, A. Hayes, F. Raynaud, S. Milhas, S. Anand, H. Tulmin, A. Miller, and T.H. Rabbitts, A cell-based screening method using an intracellular antibody for discovering small molecules targeting the translocation protein LMO2. Sci Adv, 2021. 7.

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), MSc

Intended learning outcomes:

- Primary publication in peer reviewed journals
- Patent filing with novel anti-LMO2 intracellular antibodies
- Patent filing with compound families derived from anti-LMO2 intracellular antibodies (Abd compounds)

Advertising details

Project suitable for a student with a background in:

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