The Institute of Cancer Research

Medical Research Council

The Institute of Cancer Research **PHD STUDENTSHIP PROJECT PROPOSAL**	
FUNDER DETAILS	
Studentship funded by:	Medical Research Council Industrial Collaborative Awards in Science and Engineering (MRC iCASE)
Funder specific requirements:	All MRC iCASE students will attend taught courses one day a week for the first nine months of the PhD. This training will cover computational and thematic science training as well as core and transferable skills. Students will spend the remainder of the four years on their PhD project full time with monthly cohort activities. In addition, students must spend a cumulative period of no less than three months working in the facilities of the industrial collaborator. This 3 month period can be at any point during the studentship and may consist of a number of shorter visits if appropriate.
Estimated amount and distribution of time spent with industrial partner:	6-12 months
PROJECT DETAILS	
Project Title:	**Exploring strategies for targeting oncogenic beta-catenin**
Short Project Title:	Targeting beta-catenin
SUPERVISORY TEAM	
Primary Supervisor:	Dr Paul Clarke
Industrial Supervisor:	Dr Dirk Wienke, Merck KGaA
Secondary Supervisor:	Dr Rob van Montfort
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Signal Transduction and Molecular Pharmacology

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SHORT ABSTRACT

The discovery of an inhibitor that acts at, or downstream of beta-catenin would not only be a valuable tool for exploring the role of WNT signalling in cancer biology, but importantly would address the high unmet need for better treatment options for colorectal cancer and breast cancer sub-types dependent on WNT-signalling. The project outlined here would further our biological understanding of whether transient or allosteric pockets in Beta-catenin can be targeted to displace binding of BCL9/9L an essential co-activator of oncogenic beta-catenin activity.

BACKGROUND TO THE PROJECT

Clinical and experimental data clearly indicates that some tumour types, including the majority of colorectal and triple negative breast cancers, rely on WNT signalling and oncogenic beta-catenin for initiation and progression (Clevers and Nusse, 2012; Zhan, Rindtorff and Boutros, 2017). Elevated WNT/beta-catenin signalling is also a feature of cancer stem cells isolated from different tumour types and is required to maintain their self-renewal and tumorigenic properties.

The ICR/Merck collaborative Team have considerable experience targeting WNT-signalling, including a phenotypic screening approach that discovered potent and selective CDK8/19 mediator kinase inhibitors and a biochemical screen that identified Tankyrase inhibitors (Dale et al, 2015; Menon et al, 2019).

BCL9 and BCL9L drive pathologic beta-catenin transcriptional activity that is well supported by published data and particularly studies with GEMMs. One mouse model showed that elimination of BCL9/B9L through genetic deletion in the murine gut has no overt phenotypic consequences, indicating that blockade of BCL9 function may be less harmful to normal cells compared to other WNT signalling intervention points (Deka et al, 2010}. Two later papers described the phenotype of BCL9/9L double knockout mouse models (Mieszczanek et al. 2019, Gay et al. 2019) and showed that Bcl9/9l were dispensable for most normal gene expression in normal cells but were required tumour growth in WNT-dependent tumorigenesis models. Importantly, a further study simulating pharmacologic inhibition of the BCL9:beta-catenin interaction with Bcl9/9l knock-in alleles lacking beta-catenin interaction domains, established that the phenotypes observed following complete ablation of Bcl9/9l in colorectal cancer models are mediated by the beta-catenin-Bcl9/9l interaction (Moor et al. 2015).

However, targeting the interaction between BCL9/9L and Beta-catenin is technically challenging as the interaction surface is relatively flat and features few pockets.

PROJECT AIMS

- Establish gene expression profiles for tool compounds and peptides targeting TCF4 and BCL9 binding compared with beta-catenin degradation dTAG-degradation model
- Use a rescue strategy re- expressing mutant beta-catenin to explore the impact of disrupting BCL9 and/or TCF4 on beta-catenin function, protein interactions and gene expression profile
- Use the dTAG rescue strategy to understand the importance of fragment compound binding sites for beta-catenin function and interactions using beta-catenin mutants of the fragment binding sites
- Develop and characterise CRISPR/dTAG-models for BCL9, BCL9L and TCF4 to explore the biological impact of their targeted degradation.

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RESEARCH PROPOSAL

Background

The Team is exploring the hypothesis that transient or allosteric pockets in Beta-catenin can be targeted to displace binding of BCL9/9L.

Aim

To use peptide competitors and cellular degradation models to investigate the interplay between beta-catenin, BCL9(L) and TCF4 binding and develop supporting biology evidence for the hypothesis that the cellular dependency for oncogenic beta-catenin requires BCL9 and can be targeted by allosteric binding to beta-catenin.

Project plan

a) Establish a global transcriptional profile following degradation beta-catenin of the dTAG-beta-catenin. This will establish a profile for disrupted oncogenic beta-catenin in a human colorectal cancer model (SW480). Profiles for treatment of SW480 cells with published peptide competitors of BCL9- (hsBCL9) or TCF4-binding (NLS-StAx-hs or bicyclic peptide competitor) will be used as positive controls. The activity of the peptides will be confirmed in established cellular BCL9:beta-catenin and TCF3:beta-catenin NanoBRET interaction assays and TCF/LEF promoter reporter assays. These profiles will be used as reference profiles for the remainder of the project.

b) Investigate the interplay between beta-catenin, BCL9(L) and TCF4 binding on the oncogenic function of beta-catenin determined by expressing WT or defective BCL9 and/or TCF4 binding mutants of beta-catenin in the beta-catenin degradation model and assessing their ability to rescue the impact of beta-catenin degradation. End-points will include viability, RNAseq expression profiling and profiling of beta-catenin interactors. These experiments will test the impact of disrupting TCF4-binding on the interaction of BCL9 and other proteins with beta-catenin. The dTAG rescue strategy will also be used to understand the importance of fragment compound binding sites for beta-catenin function and interactions using beta-catenin mutants of the fragment binding sites.

c) The ICR Team have recently established RNP-based CRISPR/CAS knock-in protocols to tag endogenous proteins for dTAG PROTAC degradation. This approach will be used to directly tag the endogenous BCL9, BCL9L or TCF4 gene with the mutant FKBP12^{V26F} dTAG to establish a single or double dTAG model where protein expression can be controlled by treatment with dTAG-v1 or dTAG-13. These models will be used to explore activation of oncogenic beta-catenin in human SW480 colorectal cancer cells. End-points will include viability, expression profiling by RNAseq and analysis of the beta-catenin interactome.

Outcome

We anticipate two outcomes: an understanding of the interplay between of TCF4 and BCL9 binding on oncogenic beta-catenin function and also the potential importance on the beta-catenin interactome and function.

LITERATURE REFERENCES

Clevers H and Nusse R. (2012) Wnt/β-catenin signaling and disease. *Cell*, 149(6):1192-205.

Dale T, Clarke PA, Esdar C. et al (2015) A selective chemical probe for exploring the role of CDK8 and CDK19 in human disease. *Nat Chem Biol.*, 11(12):973-980.

Gay DM, Ridgway RA, Müller M, et al. (2019) Loss of BCL9/9l suppresses Wnt driven tumourigenesis in models that recapitulate human cancer. *Nat Commun.*, 10(1):723.

Menon M, Elliott R, Bowers L, et al. (2019) A novel tankyrase inhibitor, MSC2504877, enhances the effects of clinical CDK4/6 inhibitors. *Sci Rep.*, 9(1):201.

Mieszczanek J, van Tienen LM, Ibrahim AEK, et al. (2019) Bcl9 and Pygo synergise downstream of Apc to effect intestinal neoplasia in FAP mouse models. *Nat Commun.,* 10(1):724.

Moor AE, Anderle P, Cantù C, et al. (2015) BCL9/9L-beta-catenin Signaling is Associated With Poor Outcome in Colorectal Cancer. *EBioMedicine,* 2(12):1932-43

Zhan T, Rindtorff N, and Boutros M. (2017) Wnt signaling in cancer. *Oncogene,* 36:1461–1473.

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.
Intended learning outcomes:	• Knowledge and skills required to run focused genetic screens and reporter assays • Knowledge of RNAseq analysis • Experience and skills associated with drug target validation • Exposure to state-of-the-art drug discovery at both sites

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. Wnt
	2. Beta-catenin
	3. Target validation
	4. CRISPR
	5. PROTAC
	6. dTAG