EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Development of a new approach to targeted chelation therapies

Durham University, Department of Chemistry and Pleco Therapeutics

Supervisory Team	• **Dr William D. G. Brittain, Durham University (Lead supervisor)** • **Dr Patricia A. J. Muller, Durham University (Co-supervisor)** • **Prof John Lunec, Newcastle University (Co-supervisor)** • **Pleco Therapeutics, Netherlands (Industrial Partner)**

Project overview/context

Chelation of transition metal ions has been shown to be of benefit in treating many diseases. Through the sequestering of metal ions biological pathways can be inhibited and protein folding states altered. Metal ions however are required for myriad biological processes and thus traditional chelation therapies can have unwanted side effects. By attaching a chelator to something able to target a specific biological target this would help prevent these side effects. This project will develop a new approach by attaching small molecule metal chelators to targeting peptides to minimise off target effects.

Research Project

Background:
Transition metal ion chelators can be powerful treatments for a range of diseases however these approaches can come with adverse side effects. By the conjugation of a known metal ligand with a peptide backbone, hybrid systems could be developed to generate the next generation of smart chelation therapies. This research project will develop a toolbox of peptide-metal chelator conjugates and apply them in p53 and PPM1D targeted anti-cancer therapies. To achieve these goals, the project will be split into three interlinked work packages (WP).

WP1) Synthesis of peptide-chelator conjugate library (WB Durham)
To begin small molecule chelators will be built into novel amino acids or appropriately functionalised for conjugation to larger peptides. In parallel the preparation of known p53 and PPM1D binding peptides will be conducted. New approaches to attach the prepared peptides with the metal chelators will be developed. A library of peptide-chelator conjugates will be generated. The position of the chelator along the peptide backbone will be investigated and fluorescence tags will be added to promising compounds to validate entry into cells and subcellular co-localisation with target proteins examined by immunofluorescence imaging with specific antibodies.

WP2) Evaluation of peptide-chelator conjugates to influence p53 folding (PM Durham)
Biological assays to investigate the peptide-chelator conjugate libraries ability to influence transition metals in cells will be conducted, as well as their ability to influence folding states/ function of p53. We will use luciferase reporter assays to monitor metal availability and p53 function using MRE (Metal Response Element) from MTF1 (Metal Transcription Factor 1). In addition, the peptide-chelator conjugates will be tested for their ability to enter cells using microscopy with co-localisation with p53 in the nucleus. P53 function will be monitored more closely and qRT PCR will be used to evaluate WT or mutant p53 function in the presence of these compounds and metals.

WP3) Evaluation of peptide-chelator conjugates to effect PPM1D function (JL Newcastle)

PPM1D phosphatase oncoprotein is an important negative feedback regulator of p53 and of upstream stress response kinases that signal to p53. Being causally linked with intrinsic and acquired resistance to p53-dependent therapies and emerging as a target to enhance immunotherapies. The Mg/Mn dependence of PPM1D make it a potential target for inactivation with targeted chelators based on known peptides and small molecule ligands of PPM1D. The effectiveness of conjugates will be investigated with cell-free phosphatase catalytic and binding assays, followed by intact cell evaluation for growth inhibition and cell killing using a panel of well-characterised cell lines of known PPM1D and p53 status. Evidence of on-target mechanism in intact cells will be sought by western blot and qRT-PCR analysis of p53 pathway signalling proteins and transcriptional target genes.

Project Vision:
This project will develop a new toolbox for the delivery of targeted metal chelation therapies. This platform technology could have wide implications for how chelation therapies are employed across a wide range of disease areas. This project will focus specifically on p53 and PPM1D, however we have ambitions to go far beyond these initial targets.

Further Information

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How to Apply

To apply for this project please visit the Durham University application portal to be found at: Home · Application Portal (microsoftcrmportals.com)

Training & Skills

The student will receive broad training in both chemistry and biology in Durham and Newcastle. Training in synthetic organic chemistry and specificially in the areas of small molecule and peptide synthesis. The candiate will be trainined in compound characterisation including NMR spectoscopy and mass spectrometry. Training in biological assays including how to measure metal concentrations within cells. Fluorescence microscopy training will be given to determine the localisation of compounds within cellular environments. The candidate will be given the opportunity to develop their presentation skills in regular group meetings as well as at national scientific meetings.

Please select the course ‘PhD in Molecular Sciences for Medicine (EPSRC CDT)’, which is registered in the Chemistry Department and indicate the reference **mos23_02 i**n the ‘Field of Study’ section of the application form. Please note that there is no need to submit a Research Proposal with your application, however we do require a Covering Letter, CV, academic transcripts, the contact details of two referees and proof of English language proficiency if relevant.

Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the application process at Durham University please contact the Durham MoSMed CDT Manager, Emma Worden at: emma.worden@durham.ac.uk

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