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

DUNeS: Structure based development of dual specificity inhibitors of NLRP3 and SARM1

Newcastle University (Chemistry), Durham University, RxCelerate

Supervisory Team

- **Dr Agnieszka K. Bronowska, Newcastle University (Lead)**
- **Prof Ehmke Pohl, Durham University**
- **Dr João de Souza, RxCelerate Ltd**
- **Dr Kate Madden, Newcastle University**
- **Dr Viktor Korolchuk, Newcastle University**

Project overview/context

Treatment of neurodegenerative disease needs new targets, as current approaches fail to translate in clinical trials. Elevated activation of the NLRP3 inflammasome coupled with activation of the NADase SARM1, have been implicated in early steps of neurodegeneration. Simultaneous inhibition of those two targets looks like a promising strategy to mitigate neuroinflammation.

This project will generate DUNeS: prototypical dual-specificity inhibitors of NLRP3 and SARM1, capitalising on recent structural biology discoveries. Compounds will be designed, synthesised and tested using orthogonal assay cascade, including computational, biophysical, cell-based and phenotypic assays. Once validated, compounds will provide a robust proof-of-concept for a novel therapeutic modality.

Research Project

In the early stages of neurodegeneration, microglia show elevated activation of the NLRP3 inflammasome and depletion of NAD+[1,2]. These coincide with impairment of mitophagy[3]. Dual inhibition of NAD-ase SARM1 and NLRP3 induces mitophagy more potently than inhibition of either target alone[3]. Therefore, the key objective of this project is to design, develop and test small molecules inhibiting both NLRP3 and SARM1.

SARM1 inhibition leads to an adduct formation at SARM1's orthosteric site[4]. It is feasible to expand the scaffold to accommodate a moiety which inhibits NLRP3. Our pilot data show that adduct-forming SARM1 inhibitors induce mitophagy. Incorporation of a moiety targeting SARM1 should be tolerated within the NLRP3 binding site[5]. This opens an opportunity to develop bespoke **DUNeS**: **DU**al-specific inhibitors targeting **N**LRP3 and **S**ARM1.

This studentship will contribute to developing methodology to utilise ultra-high-throughput computational workflows to develop mall molecules designed to inhibit two protein targets simultaneously. Virtual screening and subsequent optimisation were not tested for development of bispecific inhibitors yet. Such testing is much needed to allow the success of future drug development.

Research plan involves the following activities:

Data-driven discovery: The project will first assess an impact of extending the scaffold of an established NLRP3 inhibitor, MCC950, to accommodate moiety forming adducts with NAD+ within SARM1. The successful candidate will aim to optimise the NLRP3 affinity of designed small molecules, simultaneously assessing their inhibition of SARM1. The project will explore several approaches, including "manual"

[1] Hou et al, PNAS 2021,118(37):14. [2] Lawrence et al, Trends Immunol 2022,43:877. [3] Li et al., Autophagy 2022,23:1. [4] Bratkowski et al., Neuron 2022,110:3711. [5] McBride et al., J Med Chem 2022,65:14721.

structure-guided optimisation, hybrid QM/MM workflow, and screening of massive virtual libraries.

Triage, synthesis and structure-activity relationships: Synthetically feasible small molecular DUNeS will be synthesised and tested in cell-free SARM1 assay and Cas-1 NLRP3 cell-based assay. Works will include exploratory SAR studies. Compounds will be assessed on neuroinflammatory phenotypes.

Testing: Works will focus on probing the effects of lead compounds on mitophagy, NAD+ levels, Cas-1 activity, and selectivity. The most promising lead compounds will be advanced for their biophysical assays and crystal structures with TIR domain of SARM1. Compounds will be assayed for modulating liquid-liquid phase separation (in collaboration with Dr A. Wollmann, Newcastle University), effects in primary cells (collaboration with Dr R. Coll, Queen's University Belfast), and in vivo PK profiling.

Training & Skills

The successful candidate will work at an exciting interface between chemistry and biology with an input from the industrial partner, and will be rigorously trained in computational and synthetic medicinal chemistry, and cell, molecular and structural biology.
The candidate will be based in Chemistry (Newcastle), with rotations at labs in the Faculty of Medical Sciences and Durham University. Additionally, the candidate will carry out several research visits to RxCelerate (Cambridge).

The student will be trained in the following techniques:

(1) Molecular modelling, ultra-high-throughput virtual screening, hit-to-lead and lead optimisation.
(2) Medicinal and synthetic chemistry and phenotypic assays.
(3) Protein chemistry and structural biology.
(4) Specific assays (Cas-1, NAD+, mitophagy) and techniques (cell culture, cloning, lentiviral transduction, immunofluorescence), and statistical analysis.

The candidate will also be trained in biophysical assays, depending on the needs and the course of the project.

Further Information

For further information please contact the lead supervisor, Dr Agnieszka K. Bronowska
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How to Apply

If applying to a **Newcastle project**, you must apply through the University's **Apply to Newcastle Portal**. Once registered select **'Create a Postgraduate Application'.**

Use 'Course Search' to identify your programme of study:

- search for the 'Course Title' using the programme code: **8207F**
- select '**PhD Molecular Sciences for Medicine (SNES)'** as the programme of study

You will then need to provide the following information in the 'Further Questions' section:

- a 'Personal Statement' (this is a mandatory field) - upload a document or write a statement directly into the application form. Please include the full title of the studentship, the studentship code, and how your interests and experience relate to the project.
- the relevant studentship code **(mos23_03)** in the 'Studentship/Partnership Reference' field. If you wish to apply for additional studentships, please make sure to add the relevant studentship reference each time, before submitting each separate application. For example, you may wish to apply for mos23_03 AND mos23_04. **You must include the relevant code for your application to be considered.**
- when prompted for how you are providing your research proposal - select 'Write Proposal'. You should then type in the title of the relevant research project. You do not need to upload a research proposal.
- An up to date CV.
- Please upload all documents in PDF format.

Equality, Diversity and Inclusion (EDI)
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.

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Should you have any queries regarding the MoSMed application process to Newcastle University please contact Craig Hinds, the MoSMed CDT Manager: mosmed.cdt@newcastle.ac.uk

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