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

PROTAC enabled avenues for nasopharyngeal carcinoma (NPC) immunotherapy

[Durham University, Chemistry Department]

Supervisory Team	• **Professor Steven Cobb, Durham University (Lead supervisor), Professor Akane Kawamura, Newcastle University (co-supervisor) and Dr. Graham Taylor, Birmingham University (co-supervisor)**

Project overview/context

Nasopharyngeal carcinoma (NPC) is a type of cancer that affects the lining of the throat. In most cases, NPC presents at a late stage and survival is poor. Current treatments are limited to chemo-radiotherapy and are associated with significant morbidity and poor quality of life. NPC is always driven by Epstein-Barr virus (EBV) infection and this is an attractive immunotherapeutic target. Epstein–Barr nuclear antigen 1 (EBNA1), an EBV protein, is essential for the survival of infected cells. This project will develop an entirely new class of molecules that target EBNA1 and can be used for NPC immunotherapy - enabling the bodies own immune system to fight the cancer.

Research Project

EBNA1 is a key target for NPC immunotherapy but it is largely protected from antigen processing and presentation by a large internal glycine/alanine repeat (GAr) domain. *In vitro*, this protection can be overcome by genetically fusing ubiquitin to the EBNA1 sequence. In this project, we will develop ways to selectively ubiquitnate EBNA1 *in vivo*, triggering its degradation and antigenic processing.

Proteolysis targeting chimeras (PROTACs) consist of a target-specific moiety (in this work an EBNA1-targeting ligand) linked to a ubiquitin ligase recruitment ligand.[1] Recruitment of the ubiquitin ligase (e.g. E3 Ligase) to the targeted protein results in its polyubiquitination and proteasomal degradation. A PROTAC-based agent would abrogate EBNA1's pro-tumoural effects while contemporaneously rendering EBV tumour cells visible to T-cells.

Objective 1 – We will prepare a series of PROTACs by conjugating known E3 ligase targeting ligands to the linear EBNA1 peptide-targeting sequences already identified (Cobb and Taylor[2]) varying the linker size which, along with the E3 ligase ligand selected, can influence ubiquitination efficiency.

Objective 2 – We are keen to enhance the properties of our existing EBNA1 linear peptide ligands (e.g. increase proteolytic stability) and identify new cyclic peptide ligands that target this particular protein. We will use peptiod chemistry[3] to design proteolytically stable peptide-peptoid hybrid analogs of our first generation EBNA1 peptides. We will also work with the Kawamura lab, applying their cyclic peptide-based technology[4] to access entirely new types of EBNA1 specific ligands. A range of biochemical and biophysical approaches (e.g. ITC, SPR, NMR) will be used to assess EBNA1 binding.

Objective 3 – We will transduce EBNA1+ve HL lines with relevant HLA molecules and use these lines to test each PROTAC candidate, measuring cell-surface epitope peptide levels with EBNA1-specific CD8+ T-clones (Taylor lab). This assay is simple, robust and provides a direct readout of the ability of each PROTAC to enhance EBNA1's immune visibility. We will also use CD4+ T-cell clones to detect whether ubiquitinated EBNA1 can endogenously access the MHC-II pathway via p62 (SQSTM1) an autophagy adaptor protein that shuttles between the nucleus and cytoplasm, transporting ubiquitinated proteins into autophagosomes. Such access would allow NPC tumour cells, which in almost all cases are strongly MHC-II positive to be recognised by EBNA1-specific

cytotoxic CD4+ T-cells. EBNA1 contains multiple MHC-II epitopes and although able to endogenously access the MHC-II pathway endogenously via autophagy, such access is inefficient; boosting this efficiency using PROTACs is an attractive prospect.

References

(1) Cuilli A., *SALS Discovery* (2020), online https://doi.org/10.1177/2472555220965528
(2) Cobb & Taylor et al., *Nature Biomedical Engineering* (2017); 1(4), 1-10.
(3) Cobb SL et al, *J. Am. Chem. Soc.,* (2019); 141 (8):3430-3434
(4) Kawamura A et al. *Nature Comm* (2017) 8: 14773

Training & Skills

The recruited student will receive training in synthetic organic chemistry, peptide and peptoid chemistry (Cobb), peptide chemical-biology (Cobb/ Kawamura), viral & cancer immunology and immunotherapy (Taylor). Given this they will be perfectly placed at the end of their PhD to have all the required skills to work at the interface between the molecular and medical sciences.

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: https://www.dur.ac.uk/study/pg/apply/

Please select the course code F1A201 for a PhD in Molecular Sciences for Medicine and indicate the reference MoSMed21_12 in 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, an academic transcript, the contact details of two referees and proof of English language proficiency if appropriate.

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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