

## Investigating the potential of novel cyclin-dependent kinase inhibitors for therapeutic development in chronic lymphocytic leukaemia

**Drs. Alison M. Michie & Helen Wheadon**

Paul O’Gorman Leukaemia Research Centre, Institute of Cancer Sciences,  
University of Glasgow

Chronic lymphocytic leukaemia (CLL) is currently incurable with standard chemotherapeutic agents, highlighting the need for novel therapies. In addition to the deregulated expression profile of anti-apoptotic Bcl-2 protein family members resulting in CLL cell accumulation, the leukaemic clone also proliferates in response to microenvironmental signals within the lymphoid organs of CLL patients, making CLL a dynamic disease. CLL cells within proliferation centre (PCs) of patient lymph nodes (LNs) attract appreciable numbers of activated CD4<sup>+</sup> T lymphocytes expressing CD40 ligand (CD154), and interleukin 4 (IL4), which promote proliferation. Overcoming cyto-protective signals generated within the microenvironment of LNs is essential in developing a cure for CLL.

We have developed *in vitro* conditions that closely mirror the *in vivo* proliferation-promoting environment of PCs, by co-culturing CLL cells with the fibroblast cell line NT-L expressing CD154 in the presence of IL4 (CD154/IL4 system). These conditions lead to an activation of NFκB signalling and induction of anti-apoptotic proteins Bcl-x<sub>L</sub>, and survivin, mimicking the expression profile of CLL cells within patient LNs demonstrating the translational-relevance of the CD154/IL4 system for testing novel compounds for the treatment of CLL. Indeed, we recently demonstrated that the cyclin-dependent kinase (CDK) inhibitor CR8, a potent analogue of roscovitine, causes apoptosis in CLL cells in the CD154/IL4 system, which renders CLL cells resistant to the standard chemotherapeutic treatment fludarabine *in vitro*. CR8-mediated apoptosis occurred through downregulation of the anti-apoptotic proteins Mcl-1, Bcl-x<sub>L</sub> and XIAP, mediated at least in part by inhibition of RNA polymerase II, and inhibition of the classical IκBα-p65-NFκB signalling pathway. These studies indicate that CDK inhibitors represent a promising therapy for CLL. We have recently obtained seven more potent CR8 analogues (IC<sub>50</sub> < 30 nM vs. IC<sub>50</sub> = 110 nM for CR8), which we aim to characterise as potential therapies for CLL. To achieve this we will:

- (i) Test the ability of novel CDK inhibitors to elicit apoptosis in human CLL patient samples cultured in the CD154/IL4 system *in vitro*;
- (ii) Delineate the molecular mechanism utilised by the novel CDK inhibitors, assessing the deregulation of pro-survival (anti-apoptotic factors) and pro-proliferative signals (NFκB signalling), thus defining biomarkers of response;
- (iii) Define the *in vivo* response of CLL cells to the novel CDK inhibitors in a previously developed CLL mouse model.

Investigating the response of CLL patient samples to CDK inhibitors in our *in vitro* and *in vivo* experimental systems, will allow us to address the efficacy of this group of drugs in CLL, thus potentially leading to data that could inform the design of a clinical trial. A full understanding of the mechanism of response is essential to establish whether these novel CDK inhibitors could be integrated into current therapies for CLL.

This project offers an excellent training opportunity for a clinical research fellow, as our group provides an interactive research environment with both scientific and clinical staff. Indeed, the CRF will have the opportunity to interact with Drs. Mike Leach and Alison McCaig (clinicians with a primary interest in CLL), and our industrial partners during their scientific training. The project will provide exposure to a broad array of cellular and molecular techniques including: purification of CLL cells from patient samples; CLL cell cryopreservation and recovery; cell culture of cell lines and primary cells; *in vitro* co-culture systems; flow cytometry; Western blotting; RNA extraction and RT-PCR techniques; Q-PCR and Fluidigm technologies; cell drug treatment; apoptosis assays and *in vivo* mouse modelling.