3 Year PhD Studentship available for September 2019

**Department:** Genetics & Genome Biology - Leicester Cancer Research Centre

**Supervisors:**
- Dr Harriet Walter
- Professor M.J.S. Dyer
- Dr S. Jayne
- Prof D. Jones

**Eligibility:** UK/EU applicants only

**Project Title:** Barriers to CD19 therapy in B cell malignancies

**Project Description:**

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma, with an annual incidence of 3-4 per 100,000 in the UK. While most patients will respond well to immuno-chemotherapy (R-CHOP), up to 40% of patients will fail to respond or relapse within 2 years. For these patients the outcomes are usually very poor, most patients dying rapidly (median overall survival of less than 8 months) with chemotherapy refractory disease. New therapeutic strategies are needed for these patients.

CD19 is broadly expressed on the cell surface of B cells and has been explored as a therapeutic target. Different therapeutic approaches to targeting CD19 include antibody constructs and CAR-T therapies. Novel CD19 antibody drug conjugate (ADC) have shown promising activity in some patients with durable response, however it remains unknown why some patients fail to show any clinical response.

This project will aim to understand variability of response to the CD19 ADC in DLBCL utilising cell line resources, samples from our biobank, PDX models and fresh primary tissue samples. The main objectives will be to: quantify cell surface expression of the CD19 complex (CD19/CD21/CD81/CD225) in a panel of DLBCL B-cell lines, to determine rates of CD19 internalisation following exposure to bivalent CD19 antibodies and identify novel predictive biomarkers of response.

**Background**

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma. However up to 40% of patients will fail to respond to immuno-chemotherapy or relapse within 2 years. CD19 antibodies are being explored as a new therapy and this
The project will aim to understand variability of response to ADC targeting CD19 in DLBCL and identify novel predictive biomarkers of response.

**Hypothesis**

Response to therapy with anti CD19 ADC can be predicted through characterisation of the CD19 cell surface complex and CD19 internalisation studies using proteomic and genomic approaches.

**Experimental Methods and Research Plan**

Through the Ernest and Helen Scott Haematological Research Institute at the University of Leicester we have access to a large and increasingly well-characterised panel of DLBCL cell lines. In addition, the project will use the resources of the Leicester Haematology Tissue Bank and PDX models established by Dr Sandrine Jayne.

**Cell line studies.**

- Quantification of cell surface expression of the CD19 complex (CD19/CD21/CD81/CD225) in a panel of DLBCL B-cell lines using flow cytometry. An initial panel of 25 cell lines will be selected on the basis of their molecular genetic profile and on their expression of CD19 RNA. Preliminary data show marked heterogeneity in levels of expression of CD19 over at least a fifty fold range.
- To determine rates of CD19 internalisation following exposure to bivalent CD19 antibodies within this cell line panel. CD19 antibodies will be labelled with pH sensitive dyes and rates of lysosomal uptake determined. Parallel experiments have been done with CRLF2 and CD20 Mabs.
- Compare the composition of the CD19 complex in DLBCL cell lines with slow and rapid CD19 internalisation by CD19 immunoprecipitation coupled with mass spectrometry.
- So far, the activity of CD19 ADC has only been assessed on a small panel of varied malignant B cell lines. We will assess the activity of ADC in DLBCL cell lines with low and high rates of CD19 internalisation and correlate activity with the various parameters identified above.
- Measure DNA damage response (γH2AX, p53 expression) and repair (Comet assay) following treatment with CD19 ADC.
- We will compare the activity of CD19 ADC with that of free tesirine.
- We will develop CD19 ADC resistant cell lines by exposure of sensitive cell lines to increasing concentrations of the ADC. Analysis of derived cell lines will include whole exome sequencing, RNA-Seq and proteomics in order to determine key determinants of resistance.

**Studies related to CD19 ADC clinical studies in Leicester.**

Clinical trials in Leicester with ADC will enable access to fresh primary DLBCL cells and permit unique observations. We will therefore:

- Assess the activity of ADC in primary DLBCL cells in the cell bank *in vitro* and in derived PDX models.
• We will assess responses in primary cells derived from patients entering the clinical trial and compare with clinical response seen.
• Using serial ctDNA samples assess dynamic changes pre- and post-treatment *in vivo* from patients treated on trial.
• We will seek to obtain further biopsy samples from patients who either fail to respond or who develop resistance to ADC. Undertake WES and RNA sequencing of responders/non responders and following the development of resistance.

**Funding details:**

This project is in competition for a College of Life Sciences (CLS) PhD Studentship. The Studentships are for three years, starting September 2019, and offer tuition fees at UK/EU rates and a Stipend at UK Research Council rates.*

*Please note, this College of Life Sciences (CLS) PhD Studentship is joint funded by Leicester Precision Medicine Institute and local charity Hope Against Cancer.

**Entry requirements:**

Applicants are required to hold/or expect to obtain a UK Bachelor Degree 2:1 or better in a relevant subject. The University of Leicester English language requirements apply where applicable.

**How to apply:**

You should submit your application using our [online application system](#).

Apply for a PhD in Cancer Research /September 2019

In the funding section of the application please indicate you wish to be considered for a CLS Cancer Research Studentship

In the proposal section please provide the name of the supervisor and project you want to be considered for. You do not need to include a proposal but please include a personal statement giving details of why you want to be considered for this project.

**Project / Funding Enquiries:**

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**Closing date for applications**  25th February 2019