The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	**Elucidating the molecular mechanism of DNA-end resection and its role in the Double-Strand-Break (DSB) repair pathway choice.**
Short Project Title:	**Molecular mechanisms of DSB pathway choice**

Primary Supervisor(s):	Dr Wojciech Niedzwiedz
Other members of the supervisory team:	
Backup Supervisor: (must have IRS status)	Professor Chris Lord
Lead contact person for the project:	Dr Wojciech Niedzwiedz
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Genome Instability and Cancer

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

DSBs are amongst the most cytotoxic lesions a cell can suffer. Misrepair of DSBs can trigger genome rearrangements associated with inherited human syndromes with symptoms such as cancer, developmental abnormalities or premature ageing (Jackson and Bartek, 2009, Jeggo et al., 2016). DSBs are the predominant lesion driving cell death, a property that is exploited for cancer treatment. Therefore, understanding the mechanisms by which cells repair DSBs is of vital importance to develop novel cancer treatment strategies.

The two main pathways used to repair DSBs are error-prone non–homologous end-joining (NHEJ), which involves 'sticking' or joining back together the two broken ends, and error-free homologous recombination (HR), which involves a copy/paste reaction utilizing the information (DNA sequence) from an intact DNA template. The correct use of these pathways is crucial for suppression of genome instability, a potent driver of tumorigenesis. However, how cells choose between these two repair options remains poorly understood.

A key initiating step in HR is the enzymatic processing of DNA ends- DNA end-resection, which renders broken chromosomes inaccessible for the NHEJ pathway. This is a highly conserved mechanism that operates from bacteria to humans. However, despite its key role in protecting our genome, the molecular mechanism of end-resection remains poorly defined. We wish to learn more about how DSB repair is regulated in cells.

To do this we recently identified a previously uncharachterised protein, EXD2 as a novel factor

that promotes DNA end resection (Broderick et al., 2016). The aim of this PhD project will be to characterize the role of EXD2 in genome stability maintenance and tumour progression. This will answer fundamental questions about the role of chromosomal instability in cancer development. Moreover, molecular information gained regarding DSB repair pathway choice could pave the way for the development of new cancer treatments strategies.

PROJECT AIMS

***Aim 1*. A commitment to HR: defining the role of EXD2 in DSB repair pathway choice**

***Aim 2*. *In vitro* biochemistry analyses of the effect of end resection on the DNA binding of NHEJ factors.**

RESEARCH PROPOSAL

A commitment to HR: defining the mechanism of DSB repair pathway choice and the role of EXD2 in this process.

DNA end resection has an important role in initiating not only HR but also in facilitating a sub-pathway of NHEJ, termed alternative non-homologous end-joining (A-NHEJ). This mechanism of DSB repair requires MRE11/CtIP-dependent DNA resection proximal to the break site, followed by annealing of short regions of microhomology. Strikingly, A-NHEJ is highly error prone and is thought to be a major source of DSB-induced genome rearrangements in cancer cells, likely due to problems with the regulation of repair pathway choice.

Given that our preliminary data shows that EXD2 affects the kinetics of removal of proteins that promote NHEJ, **we hypothesise that EXD2 may play an important role in regulating DNA repair pathway choice.** To test this hypothesis the student will examine: 1) whether EXD2-dependent DNA processing impacts on cells' ability to direct repair towards NHEJ or A-NHEJ; 2) If EXD2 is directly required to remove or inhibit the binding of NHEJ factors.

To this end, the student will utilise state-of-the art technologies including, CRISPR/Cas9 genome editing, gene silencing, *in vitro* reconstitution of the repair process with purified proteins and high-resolution immunofluorescence microscopy.

The work outlined in this aim will increase our mechanistic understanding of how cells choose between repair pathways and how this is regulated on the molecular level and in the context of chromatin.

Expected outcomes

Overall, the work carried out by the successful applicant in this proposed PhD project will increase our basic understanding of how cells choose between DNA repair pathways in response to DNA damage. By gaining a deeper knowledge of the molecular processes that regulate DNA repair pathway choice we will identify novel druggable targets that allow us to modulate DNA repair pathway choice in cells, which could be exploited to improve cancer therapies.

LITERATURE REFERENCES
BRODERICK, R., NIEMINUSZCZY, J., BADDOCK, H. T., DESHPANDE, R. A., GILEADI, O., PAULL, T. T., MCHUGH, P. J. & NIEDZWIEDZ, W. 2016. EXD2 promotes homologous recombination by facilitating DNA end resection. *Nat Cell Biol,* 18**,** 271-280. JACKSON, S. P. & BARTEK, J. 2009. The DNA-damage response in human biology and disease. *Nature,* 461**,** 1071-8. JEGGO, P. A., PEARL, L. H. & CARR, A. M. 2016. DNA repair, genome stability and cancer: a historical perspective. *Nat Rev Cancer,* 16**,** 35-42.

CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	B.Sc. or equivalent (First or 2:1) in Biochemistry, Molecular biology, genetics.
Intended learning outcomes:	The successful applicant will learn how to 1. Plan and design properly controlled scientific experiments, informed by relevant literature. 2. Obtain a working knowledge of cutting edge molecular biology techniques such as CRISPR/Cas9 genome editing. 3. Employ state of the art microscopy techniques including the analysis of fluorescently tagged proteins expressed in human cells. 4. Gain competency in *in vitro* biochemistry techniques including DNA end resection assays. 5. Gain skills in oral presentation, with data generated presented at national and international conferences. 6. Engage in public outreach via the ICR, disseminating the important findings of the research to the general public.