PhD Project Proposal

Funder details

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Project details

Project title: Decoding the ALT Pathway: Identifying Synthetic Lethal Targets and Biomarkers for Precision Cancer Therapy

Supervisory team

Primary Supervisor: Professor Wojciech Niedzwiedz (ICR)

Associate Supervisor(s): Dr. Jadwiga Nieminuszczy (ICR)
Dr. Heike Dahmen (Merck)

Secondary Supervisor: Professor Jyoti Choudhary

Divisional affiliation

Primary Division: Cancer Biology

Primary Team: Genome Instability and Cancer

Site: Chelsea

Project background

Cancer cells must overcome the shortening of telomeres to achieve replicative immortality. While most cancers activate telomerase to maintain telomere length, up to 10-40% of cancers, particularly mesenchymal and neuroepithelial tumours, use the Alternative Lengthening of Telomeres (ALT) pathway [1]. ALT is characterized by recombination-dependent elongation of telomeres and the generation of specific markers, including C-circles and ALT-associated promyelocytic leukemia (PML) bodies [1].

Despite its prevalence in certain cancers, ALT remains poorly understood, and no targeted therapies have been developed for ALT-reliant tumours. This presents a significant challenge, particularly as ALT-positive cancers often exhibit aggressive clinical behaviour and resistance to conventional treatments [1]. Recent advances from our laboratory have implicated replication fork restart (RF) mechanisms in the ALT pathway, suggesting that these mechanisms could represent a vulnerability in ALT-positive tumours. In particular, the "choice" of RF restart pathways in ALT cells may be influenced by key nucleases and remodelers, providing a potential therapeutic avenue [2].

These characteristics make ALT-positive tumours prime candidates for synthetic lethal (SL) targeting—a therapeutic strategy that exploits genetic dependencies. Identifying SL gene-pairs and ALT-specific biomarkers could enable precision treatments for these difficult-to-treat cancers.

To address these challenges my lab is developing an innovative machine learning-based bioinformatics pipeline that integrates whole genome sequencing (WGS) data to identify novel SL interactions and ALT specific biomarkers. This approach leverages computational power to screen large datasets, in order to reveale vulnerabilities in ALT-reliant tumours that can be therapeutically exploited. By doing so, we aim to offer new avenues for patient stratification and targeted intervention.

In summary: This research has the potential to identify actionable therapeutic targets, transforming the treatment landscape for a significant group of cancer patients.

Project aims

- **Identify and validate synthetic lethal targets in ALT-reliant cancer cells using bioinformatics-driven approaches.**
- **Characterize key ALT-specific biomarkers for diagnostic and therapeutic applications.**
- **Investigate the molecular mechanisms behind synthetic lethality in ALT-specific gene-pairs.**
- **Develop and test ALT-specific therapeutic strategies using in vitro and in vivo models.**

Research proposal

Aim 1: Identification and Validation of Synthetic Lethal Targets in ALT-Positive Cells

The first stage of this project will focus on **benchmarking** top hits from our bioinformatics pipeline and mass spectrometry-based screen, which mapped proteins specifically enriched on chromatin in ALT-dependent cancers following DNA damage. This approach will help identify and prioritize synthetic lethal (SL) interactions unique to ALT-reliant cancers. We will utilize whole genome sequencing (WGS) data from Genomics England, concentrating on tumors with a high ALT prevalence, such as sarcomas and gliomas.

Experimental Design: The WGS-based pipeline will highlight candidate SL gene-pairs based on genetic co-dependencies in ALT-positive cells. We will prioritize gene-pairs for which we observe high enrichment on chromatin upon DNA damage in ALT-reliant cancers, as these are likely required for DNA damage repair.

Methods: Top hits from bioinformatics and MS screening will be validated in ALT-reliant cell lines (e.g., U2OS, SaOS-2) using CRISPR-Cas9 knockout and/or RNAi silencing techniques. Proliferation and cell survival assays will be employed to confirm SL interactions, as we have done in prior studies [2].

Expected Outcome: The identification of critical SL gene-pairs specific to ALT-reliant cancers, laying the foundation for further exploration of therapeutic vulnerabilities.

Aim 2: Characterization of ALT-Specific Biomarkers for Clinical Application

In tandem with SL target identification, we will use whole genome sequencing (WGS) data to identify ALT-specific biomarkers that can serve diagnostic or therapeutic purposes. These biomarkers could improve patient stratification and guide clinical decisions in treating ALT-reliant tumours.

Experimental Design: We will analyse WGS data from ALT-positive tumours, particularly focusing on common drivers, mutational signatures, and genome-wide insertions of telomeric sequences, to identify potential biomarkers associated with the ALT pathway. Candidate biomarkers will be validated in silico using WGS data from ALT-reliant cancer cell lines, with the goal of developing ALT-specific diagnostic tools. We will also explore the role of these biomarkers in telomere maintenance, focusing on their correlation with telomere length and C-circle formation.

Expected Outcome: The identification and validation of WGS biomarkers that can be employed for diagnostic purposes and targeted therapies in ALT-positive tumours.

Aim 3: Investigation of the Molecular Mechanisms Underlying Synthetic Lethality in ALT

Following the identification of key SL gene-pairs, we will explore the molecular mechanisms behind the observed SL interactions, focusing on how these interactions affect telomere maintenance, replication fork restart and survival in ALT-reliant cells.

Experimental Design: We will investigate how loss-of-function mutations in SL gene-pairs affect replication fork restart and activation of DNA repair pathways in ALT-positive cell lines. This will involve CRISPR-mediated knockout/silencing of identified SL targets, followed by characterization of telomere elongation dynamics, activation of ALT-specific markers such as APBs, formation of DNA-double strand breaks at telomeres, kinetics of DDR responses, and survival; as we have done in prior studies [2].

Methods: Telomere dynamics will be analysed using CO-FISH and DNA combing techniques, while replication stress and DDR will be assessed using DNA fibre assays and immunofluorescence, as previously performed [2, 3]. Additionally, immuno-FISH will be employed to localize ALT-specific proteins at telomeres.

Expected Outcome: A comprehensive understanding of the molecular mechanisms driving synthetic lethality in ALT-reliant cells, informing the development of targeted therapies.

Aim 4: Development of ALT-Specific Therapeutic Strategies

The final phase of the project will focus on developing therapeutic strategies to exploit the vulnerabilities identified throughout the study. The penetrance of ALT-specific targets will be tested using an extended collection of validated ALT-reliant cell lines to determine their potential efficacy in treating ALT-positive cancers.

Experimental Design: We will utilize siRNA or CRISPRi against validated SL targets in ALT-reliant cell lines. Additionally, combination treatments targeting both SL targets and DDR pathways will be tested for potential synergistic effects.

Methods: Cell viability assays, drug synergy experiments, and DDR activation assays will be employed to evaluate the efficacy of candidate therapies, as done in recent studies [2].

Expected Outcome: The identification of effective ALT-specific therapeutic strategies, potentially leading to clinical trials for treating ALT-reliant tumours.

Impact and Relevance

This project will leverage a bioinformatics-driven approach to address a critical gap in cancer therapy by identifying synthetic lethal targets and biomarkers specific to ALT-reliant tumours. By integrating computational analysis with experimental validation, this research aims to develop novel diagnostic tools and therapeutic strategies that could transform the clinical landscape for patients with ALT-positive cancers. The outcomes of this project will not only provide a deeper understanding of the ALT pathway but also offer precision-targeted treatment options for some of the most aggressive and treatment-resistant cancers.

Benefits of Industry Collaboration:

This project presents a unique opportunity for collaboration with industry, which can accelerate the development and validation of new biomarkers and therapies for ALT-reliant tumours. By partnering with industry in bioinformatics and drug development, this collaboration will facilitate access to cutting-edge technologies, resources, and expertise that can enhance the project's translational potential. The integration of industrial knowledge will help fast-track the creation of diagnostic tools and therapeutic strategies, enabling the rapid transition from bench to bedside. Additionally, such partnerships can open pathways for clinical trials and commercialization, offering a competitive edge in the evolving oncology landscape while addressing a critical gap in cancer treatment.

Literature references

[1] . MacKenzie D., et al. ALT Positivity in Human Cancers: Prevalence and Clinical Insights. Cancers, 2021.
[2] 2. Broderick R., et al. Pathway choice in the alternative telomere lengthening in neoplasia is dictated by replication fork processing mediated by EXD2's nuclease activity. Nature Comm. 14, 2023.
[3] 3. Nieminuszczy J., et al. EXD2 Protects Stressed Replication Forks and Is Required for Cell Viability in the Absence of BRCA1/2. Molecular Cell 2019.

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: B.Sc. or equivalent (First or 2:1) in Biochemistry, Molecular biology, genetics, bioinformatics

Intended learning outcomes: During the course of the project, the student will become proficient in a broad range of techniques, including CRISPR-Cas9 genome editing, super-resolution fluorescence microscopy, single molecule analysis of DNA replication, bioinformatics and proteomics. The student will be encouraged to attend and present their work at national and international meetings, and to be involved in organizing and presenting at journal clubs and internal seminar series.

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Advertising details

Project suitable for a student with a background in:

- [X] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [X] Computer Science