Progress report form Cancer Council ACT Research Grant Please submit electronically to cancer.information@actcancer.org		
Report due date	13 December 2024	
Project Lay Title	Developing new therapies for B-cell lymphoma	
Grant Amount	$75,000	
Chief Investigator	Rachel Woodhouse	
Project dates	Start: 1 July 2024	End: 30 June 2025

Project Aims/Objectives Briefly state the aims from your original application.	We aim to characterise the molecular mechanisms underlying tumour cell killing by Menin and EZH2 inhibitors in Diffuse Large B Cell Lymphoma (DLBCL), to develop Menin inhibition as a novel therapeutic for DLBCL as a monotherapy or in combination with EZH2 inhibition. This will be achieved through the following 3 aims: 1) Identify molecular targets driving inhibitor response and resistance using CRISPR-Cas9 knockout screening. 2) Validate top screen hits and elucidate mechanisms of Menin and EZH2 inhibitor-mediated tumour growth inhibition in DLBCL. 3) Test a range of Menin inhibitors to identify the most potent and on target for in vivo studies and support identification of the most appropriate drug for clinical trials.
Please describe how the project is progressing against the aims. Word limit is approximately 400 words.	We previously performed a whole genome CRISPR screen in a human EZH2-mutant DLBCL cell line treated with Menin inhibitor and EZH2 inhibitors, individually or in combination. This screen revealed clinically relevant drug resistance mechanisms and novel combination therapy targets. Due to the promising nature and clinical relevance of the hits from this initial screen, we prioritised following up these hits with validation and mechanistic experiments (Aim 2). Additional CRISPR screens (Aim 1) are scheduled for January 2025 and will be completed by March. Aim 2: Our resistance screen highlighted cell cycle regulator disruption as a key resistance mechanism to both inhibitors. We selected the top 4 targets mediating resistance to inhibitors and have successfully validated these across six different EZH2-mutant DLBCL cell lines. One of the top hits was the cell cycle progression gatekeeper Rb (encoded by the RB1 gene), and another was a component of an E3 ligase complex which regulates Rb cell cycle progression ("EL1"). Using cell cycle assays, biochemical approaches, and transcriptional profiling, we have shown that Menin and EZH2 inhibitors induce cell cycle arrest, and that loss of RB1 or EL1 overcome inhibitor-mediated cell cycle arrest by bypassing cell cycle checkpoints. Patient cohort data reveals that mutations in cell cycle regulators are frequent in DLBCL, potentially explaining the poor response to EZH2 inhibitors observed in clinical trials. Notably, combining Menin and EZH2

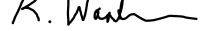
	inhibitors enhances growth inhibition and overcomes resistance, offering a promising strategy for patients with such mutations. Our sensitisation screen identified promising candidates driving sensitisation to both EZH2 and Menin inhibitors. Validation of the top 5 targets is underway and will be completed by Christmas. Mechanistic studies on validated hits will be pursued in 2025 to develop combination therapies that boost therapeutic responses. To further dissect drug response mechanisms, we performed RNA-seq on three DLBCL cell lines at days 1, 3, and 5 of treatment. Initial analysis revealed shared pathways, including type I interferon upregulation and cell cycle pathway downregulation. CUT&Tag profiling of histone modifications under inhibitor treatment is ongoing, with unbiased analyses underway to identify key mediators of drug response. CUT&RUN experiments will be performed in early 2025 to continue profiling the mechanisms of drug response. Aim 3: We evaluated four different Menin inhibitors for efficacy and specificity across a panel of EZH2-mutant and wild-type DLBCL cell lines. EZH2 mutant lines consistently responded to Menin inhibitors, with Ziftomenib (KO-539) emerging as the most potent. This inhibitor is now prioritised for ongoing studies and supports its use in future in vivo studies and clinical trials for DLBCL.

Moderating Issues Please describe any challenges that you are currently facing and/or that may have impacted upon intended activity, progress and outcomes. Please explain how you will mitigate against these circumstances. Word limit is approximately 300 words.	Our sensitisation CRISPR screen identified several promising hits which augment inhibitor response. Validation assays across a panel of DLBCL cell lines revealed that one top hit is specific for the cell line in which the screen was performed, and did not validate across other cell lines in the panel. We will therefore de-prioritise following up this hit as it may be less clinically relevant across a broad spectrum of patients. We are currently performing validation of an additional four sensitisation hits, with the aim of identifying targets which validate broadly across our panel of cell lines. Our additional CRISPR screens (scheduled for January 2025) will also enable to us to identify sensitiser hits which are common across cell lines. Such hits represent potential targets for new combination therapies to improve patient response.

Publications and presentations Please list any publications and/or abstracts produced as a result of the project. Include manuscripts in	CI Rachel Woodhouse presented this project at the following meetings: - Oral presentation at Biomolecular Horizons 2024 - "Menin inhibition as a novel epigenetic therapy for EZH2-driven diffuse large B-cell lymphoma" (September, Melbourne) - Poster presentation at the Garvan International Signalling Symposium 2024 – "Menin inhibition as a novel epigenetic therapy for EZH2-driven diffuse large B-cell lymphoma" (October, Sydney). **Awarded Best Poster Prize**

preparation or in submission/under review.	- Oral presentation at Canberra Hospital Haematology CPD meeting – "Epigenetic Therapies for Diffuse Large B Cell Lymphoma" (Canberra, November) Elizabeth Mee, our PhD student on this project, was selected for the following conference presentations: - Oral presentation at the Canberra Health Annual Research Meeting (CHARM) 2024 - Oral presentation at the John Curtin School of Medical Research Student Conference 2024 - Poster presentation at the EMBL Australia PhD Course 2024 (Melbourne, November) We are preparing a manuscript from this project for submission for publication mid next year, co-first-authored by Rachel Woodhouse and Elizabeth Mee.

Other Comments Please outline any other items of general interest which have arisen as a result of the project.	We are immensely grateful to CCACT for supporting our work on developing new treatments for lymphoma. This work would not be possible without the generous support of CCACT and the CCACT network of fundraisers and supporters.

Signed Chief Investigator	
Date	13/12/24