Final report form Cancer Council ACT Research Grant Please submit electronically to cancer.information@actcancer.org		
Report due date	By 30 August 2024	
Project Lay Title	Understanding how FUBP1 drives primary brain cancer towards rational drug discovery	
Grant Amount	$75,000	
Chief Investigator	Olga Zaytseva	
Project dates	Start: 01/06/2023	End: 31/05/2024

Project description Please explain the purpose of your research (including background and rationale). Please use language that the general public will understand. Word limit is approximately 250 words.	Our project aimed to characterise oligodendroglioma, a subtype of stem cell driven glioma, predicted to be driven by FUBP1 mutations. Our Drosophila genetic studies demonstrated that FUBP1 loss drives neural stem cell expansion in the brain, which has significant implications for brain tumour initiation. Therefore, we pursued a two-fold approach to investigate how FUBP1 might drive human glioma initiation and progression. First, to determine whether FUBP1 mutations correlate with cancer progression, primary glioma cell lines, derived from patients treated at the Canberra Hospital and deposited in the ACT Brain Cancer Biobank, were analysed for tumour characteristics (e.g. cell growth, cell division and differentiation). To further determine the significance of FUBP1 mutations in initiating glioma, we wanted to compare either FUBP1 wild type or FUBP1 mutant 3D culture systems, derived from induced pluripotent stem cells (iPSC), to generate brain organoids (mini-brains). As the mini-brains more closely resemble the normal brain environment, this system will provide a more reliable model to understand how FUBP1 loss drives glioma.

Major results of this research project As this project is now complete, please explain the major results of your research, and what it means for advancing cancer control. Please use language that the general public will understand. Word limit is approximately 500 words.	With CCACT support, we are leveraging our clinical connections, through the Canberra Brain Cancer Collaborative and the ACT Brain Cancer Biobank, to establish glioma stem cell cultures for expansion and next-generation genomic sequencing. We have optimized cell culturing methods to enable 14 glioma stem cell lines to be expanded from consenting patients for genomic sequencing. As part of proposed studies, analysis of 4 lines has identified an FUBP1 wild type oligodendroglioma line. Our follow up analysis confirmed expression of FUBP1 protein and stem cell markers in our oligodendroglioma cells cultured in neural stem cell selection media. Therefore, the FUBP1 wild type glioma line will enable further studies into the significance of FUBP1 mutations to tumour progression. Moreover, the generation of FUBP1 loss-off-function mutations in an isogenic background will enable direct comparison of cell viability, growth and cell fate markers i.e. markers of tumourigenesis. Given that the precious patient glioma samples can only be subject to limited expansion in the lab, we sought to first optimize the FUBP1 CRISPR-

	based mutagenesis strategy using an alternate, commercially available wild type induced pluripotent stem cell (iPSC) line. We have successfully induced formation of 3D brain organoids using these iPSC lines, which contain neural stem cell, progenitors, and maturing glia and neurons. Therefore, according to the aims of the project, we will next use CRISPR to perform specific knockout of FUBP1 in the iPSC cells, to model loss-of-function of FUBP1 to recapitulate mutations observed in human patients. We have obtained the necessary CRISPR reagents including FUBP1-targeting gRNA and Cas9, for mutagenesis of iPSC cells, which is currently under optimization. The successful generation of FUBP1-null iPSC cells will inform the CRISPR strategy for generating the same FUBP1 loss-of-function mutations in our patient glioma cell lines. Analysis of the growth and proliferation of wild type and FUBP1-null 3D neural brain organoids, derived from either glioma patient samples or iPSC lines, will reveal the capacity of FUBP1 mutation to drive glioma, and in future will provide a model to identify drugs that target glioma stem cells while leaving healthy/wild type brain tissue intact. We will also investigate whether the findings from our fly models can be translated to human models, for example the key molecular pathways that are regulated by FUBP1, for a deeper understanding of glioma biology. Together, our studies are generating human preclinical models for FUBP1-mutant driven glioma, which in the future will form basis of drug screening pipelines toward developing novel therapies for glioma and ultimately improve patient outcomes.

Moderating Issues Please describe any challenges that you faced and/or that have impacted upon intended activity, progress and outcomes. Please explain your strategies for any aspects of the project that are incomplete. (Limit 300 words)	One of the primary challenges that has impacted progress on the project was that the start date coincided with my return from parental leave, and ongoing part-time employment, which has limited my time commitment to the project. Nevertheless, the project has spurred a new collaboration with a team of bioengineer researchers, with expertise in generating 3D human brain organoid models. As a result, we have engaged a PhD student to complete several key aspects of the work, which will enable the successful completion of the research aims. Moreover, based on feedback from experts in the field, and our own observations, we have improved our technical approaches through protocol optimization. Thus, our adapted methodology will enable us to advance our studies to more readily complete the aims of the proposal. This will include improved CRISPR-based mutagenesis of FUBP1 in iPSC lines, to increase the chances of successfully generating FUBP1-null isogenic patient derived glioma cell lines, as outlined in our aims.

Publications and presentations Please list any publications and/or abstracts produced as	Manuscripts in preparation Nan-hee Kim, Tanya Javaid, Naomi Mitchell, Caroline Delandre, Ross Hannan, David Levens, Owen Marshall Leonie Quinn*, and Olga Zaytseva*, (*co-senior, co-corresponding author). “FUBP1/Psi represses proliferation and cell growth in the neural stem cell lineage.”

a result of the project. Include manuscripts in preparation or in submission/under review.	Damien Muckle, Brooke Kinsela, Naomi Mitchell, Caroline Delandre, Eduardo Eyras, Ross Hannan, David Levens, Owen Marshall Leonie Quinn*, and Olga Zaytseva*, (*co-senior, co-corresponding author). "FUBP1/Psi function in the niche is essential to prevent neural stem cell overproliferation." Conference presentations Biomolecular Horizons 2024, Melbourne. 2 oral presentations and 2 poster presentations from the team, related to the project.

Further studies and/or funding Please outline any further studies or funding which have arisen as a result of the project.	This project has supported collaborative projects involving clinical connections through the Canberra Brain Cancer Collaborative. This comprises projects by clinicians to investigate molecular aspects of glioma, in collaboration with wet lab researchers at ANU.

Other Comments Please outline any other items of general interest which have arisen as a result of the project.	

Signed Chief Investigator	
Date	30/8/24