Research Grants 2025

Brain tumour | A ddPCR-based ctDNA Liquid Biopsy for Glioma

Associate Professor Andrew Morokoff

Gliomas, the most common brain cancer remain a devastating diagnosis, disproportionately affecting young people and despite best treatment with surgery, chemotherapy and radiotherapy, they have a universally dismal prognosis.

A circulating molecular biomarker (known as a "liquid biopsy") does not currently exist for brain tumours but is desperately needed. Liquid biopsies could circumvent the high-risk neurosurgical procedures traditionally needed to access biopsy tissue. Furthermore, surgical tissue biopsies which are only tiny pieces of the whole tumour, can be subject to sampling error, whereas a blood test is more able to give a complete picture of the wholistic gene mutation profile.

Our research group focuses on developing a liquid biopsy for glioma based on circulating tumour DNA (ctDNA) in plasma (from blood tests). ctDNA has the advantage of extremely high specificity for gliomas, because the known mutations in the tumour can also be detected in the blood and therefore used for diagnosis or monitoring.

A very exciting update is that our work over the last 2 years, with the assistance of the RMH Neuroscience Foundation grant funding, has culminated in 2 published papers in 2024 in Neuro-oncology Advances.

Firstly, we found that using ddPCR we can detect key DNA mutations in the blood of glioma patients including IDH mutations in low grade glioma. Second, using Next Generation Sequencing (NGS) techniques on blood (AVENIO) from high grade gliomas, we reported the early detection of DNA mismatch repair mutations caused by chemotherapy leading to resistance. This is a first in the world discovery that is garnering a lot of international attention and offers of collaboration. For instance, we have recently been invited to collaborate to provide our expertise on glioma ctDNA to a prospective trial in the UK and also to research with the University of Toronto.

We now plan to extend our work significantly and validate our ctDNA tests clinically. Over the last 6 months we have been prioritising ddPCR for IDH1 mutation for the ANHEART Phase 0 trial running at RMH (PI – Jim Whittle) and this has allowed us to optimise our ddPCR methods and techniques which are working well. This further funding allows us to focus on expanding the ddPCR work including IDH1, IDH2, TERTp and EGFRvIII as well as novel mismatch repair mutations in glioma (MSH2/6) mentioned above to the whole of our stored biobank samples.

Grant $72,000

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Progress Report

1. Development of a Glioma specific sequencing panel

In collaboration with Dr Stephen Wong at PMCC, we have developed a glioma-specific circulating tumour DNA (ctDNA) sequencing panel covering 100 clinically relevant genes, including the TERT promoter, IDH1, and other key glioma drivers. Analytical validation using reference materials demonstrates sensitive mutation detection down to 0.4% VAF with as little as 10 ng of input DNA. Applying this panel to 84 patients from the Royal Melbourne Hospital Brain Collection, we achieved an overall ctDNA detection rate of approximately 21%. This is consistent with previously published studies—which were generally limited by small sample sizes—and our dataset represents the most up-to-date and largest analysis of its kind, further validating earlier observations. As expected, detection rates increased with tumour grade, with frequent identification of TERT promoter mutations and no detectable IDH1 mutations in plasma.

2. Detection of ctDNA with ddPCR

In addition, we performed IDH1 digital PCR on plasma samples from 11 patients enrolled in the AnHeart clinical trial. IDH1-mutant ctDNA was detectable in 83% of pre-treatment samples, and in several cases, ctDNA dynamics closely mirrored each patient's clinical disease course.

Both of these projects are in manuscript preparation for submission to scientific journals soon.

2. Detection of ctDNA with ddPCR

Our research has had knowledge and dissemination impact in 2024-25 with publications and talks. We published two papers in 2024 in the international key opinion leading journal Neurooncology Advances. The first paper detailed the use of the commercially available AVENIO (Roche) ctDNA panel in 10 patients showing ability to detect key glioma mutations in plasma, including MSH family mutations associated with temozolomide resistance (DOI: 10.1093/noajnl/vdae027, 17 citations). Our second paper showed, using digital droplet PCR (ddPCR) that IDH1, TERT and EGFR mutations could be detected in plasma from both low and high grade glioma (DOI: 10.1093/noajnl/vdae041, 11 citations).

This work was presented in multiple local and international meetings, most notably by A/Prof Morokoff in the Eccles Lecture 2024 at the Neurosurgical Society and Neuroscience Society meeting of Australia.

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Progress Report

4. Collaborations and networking

Our liquid biopsy work has resulted in much interest in Australia and internationally and the kick start funding of $200k from the Brain Cancer Centre has enabled the project to flourish and achieve milestones.

- As part of the BCC in Parkville, the ctDNA program is discussed regularly as part of the GLIMMER and BrainPOP research platforms.
- We are members of the UK and US-based Liquid Biopsy Consortium, most recently met at Society of Neuro-oncology (SNO) meeting in Hawaii in Nov 2025, and planning collaborative research in ctDNA methylation and others.
- We have developed collaborations with the liquid biopsy lab at Mayo Clinic, Rochester MN run by Dr Terry Burns (Neurosurgery) to discuss analysing serial CSF samples.
- We have established collaborations with Australian groups interested in blood samples and liquid biopsy, most notably the Mark Hughes Foundation and Hunter Research in Newcastle. Sample sharing agreements will be set underway in 2026.
- Initiated discussions with Ryan van Laar from Geneseq to collaborate with their miRNA-based platform which is in commercial use for Melanoma, but has translational aspects to glioma
- Applied for NHMRC Ideas Grant 2026 for ctDNA in glioma project, but unfortunately unsuccessful. Will pivot to other grant applications next year.
- Ongoing funding support for the ctDNA project from the RMH Neuroscience Foundation – $70k per year for which we are deeply grateful.

5. Teaching and students

The project has supported several research students over the past few years.

Recent students include:

Dr Jordan Jones
PhD MBBS FRACS (Neurosurgeon)
2020-2022 'A ctDNA based liquid biopsy for glioma'

Dr Kieran Benn
MD student 2024-25 and current MPhil student
Working on the ddPCR project

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Progress Report

6. Consumer Engagement

A key component of the development of our research strategy has been the early and ongoing involvement of consumers in the conceptualisation and implementation of the project. Together with our collaborators at WEHI, the WEHI Consumer Buddy Program invited four consumer representatives to provide comment and direction on the project. The potential of a glioma liquid biopsy to improve quality of life for patients and to inform novel clinical trials was extremely positively viewed by the consumer group. A consumer advisory reference group continues to play a leadership role in our combined glioma research programs (GLIMMER, BrainPOP) including the ctDNA liquid biopsy program.

RESEARCH PLAN 2026

Our next phase of work currently ongoing is further analysing the ability of ddPCR to find mutations of interest in our new cohort of 300 patients. From this we will obtain detailed information about statistical sensitivity and specificity that can be incorporated into clinical trial planning. We have been fortunate to have a new BioRad ddPCR machine loaned to our lab on a research agreement, that is able to do 'multiplex' PCR – i.e. analyse for up to 7 different mutations at the same time, making this analysis faster and more accurate. We are optimistic about the future and making further positive impact on the lives of brain cancer patients, whilst promoting the work of the Royal Melbourne Hospital Neuroscience Foundation.