Progress Report

Author: Dr Jordan Jones
Qualification: MBBS
Institution: Melbourne University/Royal Melbourne Hospital
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Title of Project: Circulating Biomarkers in Glioma

Summary:
Gliomas are the most common primary brain cancer, with approximately 2000 Australians diagnosed each year, and its most aggressive form, glioblastoma (GBM), is rapidly uniformly lethal. Circulating biomarkers have the potential to aid in a number of challenges that are faced in managing these patients and could be implemented in all phases of care (Fig. 1). Despite this currently there is no validated liquid biopsy test for glioma despite significant progress seen in other cancer types. With the support of the Brain Foundation, we have investigated the potential of circulating serum microRNAs (miRNA) to provide a more accurate assessment of tumour burden and prognosis when compared to current standard methods (MRI). Currently, we have identified a highly accurate diagnostic miRNA "signature" and found individual miRNAs that correlate to tumour burden on MRI, leading to a publication of these results. Furthermore, we have identified a 6-gene miRNA signature that can predict early progression and poor overall survival at the time of diagnosis. Validation of this work has been commenced and all experimental work completed in 119 prospectively collected patients diagnosed with glioma.

Hypothesis vs Findings
Hypothesis: We hypothesize that tumour specific microRNA is secreted from gliomas into the blood stream, and that their detection can be used to improve the clinical decision making for this group of patients.

Overall Aim: Assess and validate identified candidate circulating biomarkers of progression and survival in a prospective study of glioma patients (n=200+)

Recruitment: The prospective validation cohort (n=119) has been recruited from patients with a diagnosis of glioma admitted to The Royal Melbourne Hospital. Pre-operative blood samples, tumour tissue and postoperative bloods are taken during their initial hospital admission. Follow up bloods are taken at each follow-up MRI or at attendance to the neuro-oncology outpatient clinic with a total of >350 follow up samples. Recruitment was ceased for 3-4 months (April 2020 – July 2020) when the University of Melbourne Research labs were closed due to the COVID-19 pandemic.

Aim 1: Investigate and validate a serum microRNA signature for tumour monitoring in the post-operative period as a minimally invasive and more accurate predictor of progression

Findings:

As previously reported, we have identified a 9-gene miRNA signature that could distinguish between glioma and healthy controls with 99.8% accuracy. Two miRNAs miR-223 and miR-320e, best demonstrated dynamic changes that correlated closely with tumour volume in LGG and GBM respectively in 10 patients with multiple follow-up samples (Fig 2 and 3). Mean absolute concentrations of miRNA were higher at the time of progression compared to stable disease and miRNA concentrations were not elevated in the setting of tumour pseudo-progression, suggesting that circulating miRNA levels have a direct relationship to intracranial tumor load (Fig 4.). The data and work from this aim was published as the first blood-based miRNA monitoring paper in glioma (Morokoff A, Jones J et al. Serum microRNA is a post-operative monitoring tool in glioma. *Journal of Neuro-oncology. 2020 Sep;149(3):391-400).* We have commenced validating this work in our prospective cohort and have completed ddPCR of each of the 9-miRNA including in our diagnostic signature. Preliminary data suggest increased concentrations of miR-320e and miR-223 in gliomas, compared to healthy controls (Fig 5.). The next step in this project is to compare the MRI's in the follow up period to the miRNA concentrations which we expect to be completed in the next 2-3 months.

Aim 2: Identify and validate a serum microRNA biomarker for prognostic predictions at the time of first diagnosis in order to guide adjuvant therapy decisions.

Findings:

We analysed the 800-miRNA profile data from preoperative serum samples from our initial 91 cohort. Due to the high dimensionality of the data, we used a lasso regression model to identify best predictive miRNAs of overall and progression free survival in additions to the classical predictors age, sex, IDH-1 mutation and WHO grade. We identified the top 6 miRNAs (miR-485, 548, 20b, 444-3, 18b, 1258) that were associated with survival. The 6 gene miRNA signature was able to divide the cohort into high risk and low risk of both overall and progression free survival (Fig 6.). We have created a clinical nomogram that incorporates the included miRNAs with the other known survival predictors and are in the process of validating this with our prospective cohort as the survival data matures. A manuscript for this work is in preparation.

Presentations:

- *Melbourne Brain Centre Research Symposium - 2019*
- *Royal Melbourne Hospital Research Week – 2019*

- *Congress of Neurological Surgeons Annual Scientific Meeting – San Francisco 2019*
- *American Association of Neurological Surgeons Annual Scientific Meeting – Boston 2020 – Accepted for oral presentation however cancelled due to COVID*
- *Journal of Neuro-Oncology Webinar "Tumour Talk". Invited talk. Live Virtual. October 2020*
- *North American Society of Neuro-Oncology Annual Scientific Meeting. Virtual. November 2020*
- *North American Society of Neuro-Oncology Annual Scientific Meeting – Round table expert panel presentation and discussion. Live Virtual. November 2020*
- *Annual Academic Surgery Conference 2020. RACS. Virtual. November 2020*
- *University of Melbourne Graduate Research Conference 2020. Virtual. December 2020*

Publications:

- *Morokoff A, **Jones J** et al. Serum microRNA is a biomarker for post-operative monitoring in glioma. Journal of Neuro-oncology. 2020 Sep;149(3):391-400*
- ***Jones J** et al. Circulating Biomarkers in Glioma: A Review. Neurosurgery. 2021 Jan 13:nyaa540*
- ***Jones J** et al. A 6-gene miRNA signature for prognostic prediction in gliomas. In preparation for submission to Neuro-Oncology*

Unanswered Questions

These results require ongoing validation in our prospective cohort. If they are confirmed then the next step is to plan a clinical trial involving the use of our miRNA biomarker in assisting clinical decision making, like has been done in other cancer types. In particular, we can imagine this biomarker having significant impacts in the following;

1. Accurate **diagnosis** – particularly in tumours located in eloquent regions where biopsy may be high risk for procedural morbidity.
2. **Prognostic** predictions to accurately determine timing of additionally therapy.
3. **Monitoring** for tumour recurrence and differentiating between true tumour progression and pseudo-progression as a result of treatment.

We have also been investigating the potential of circulating tumour DNA as a biomarker in our cohort of gliomas.

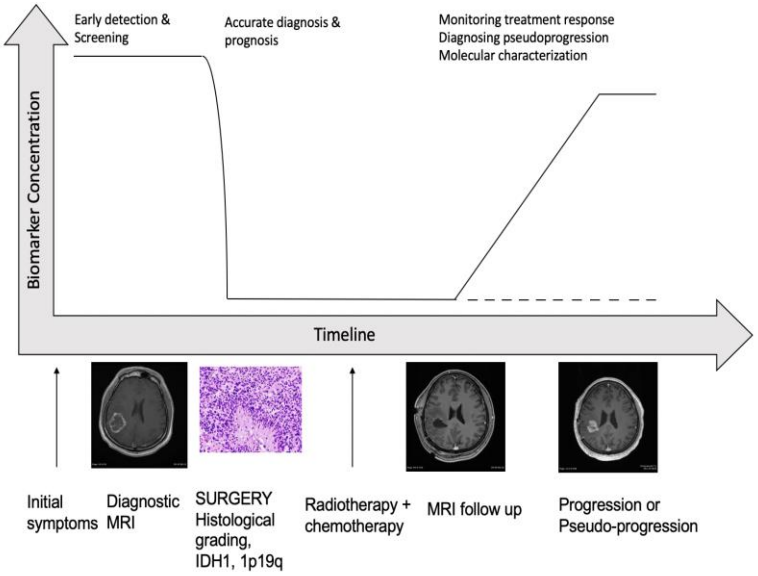

Fig 1. Schematic timeline of glioma patient diagnosis, therapy and standard monitoring on MRI.
Liquid biopsy concentration indicated on y-axis with clinical utility for prognostic classification, molecular characterisation, monitoring of treatment response and diagnosis of progression vs pseudoprogression at follow up.

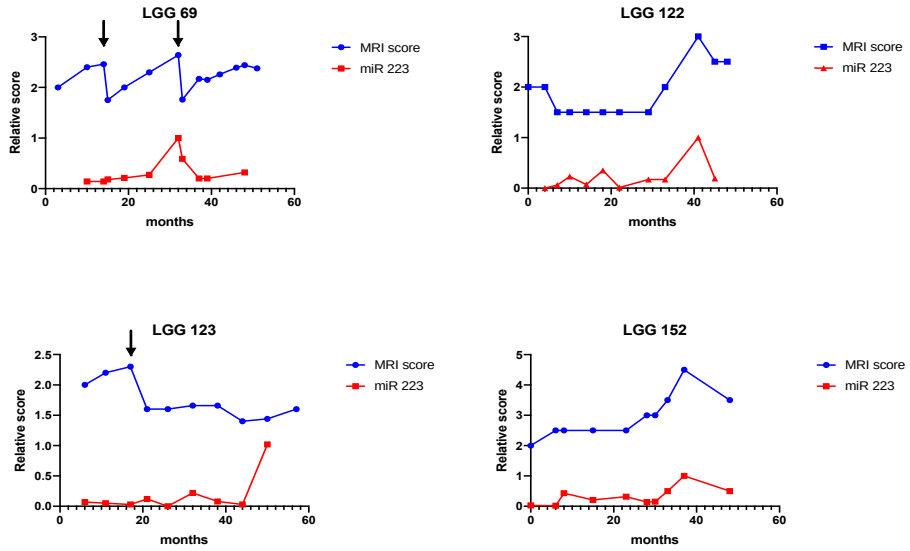

Fig 2. MiRNA-223 as a monitoring biomarker in low grade glioma. Diagrammatic timeline charts showing months on x-axis and relative miRNA concentration (orange) and MRI tumour volume (blue) on y-axis. Arrows indicate timepoints of surgery.

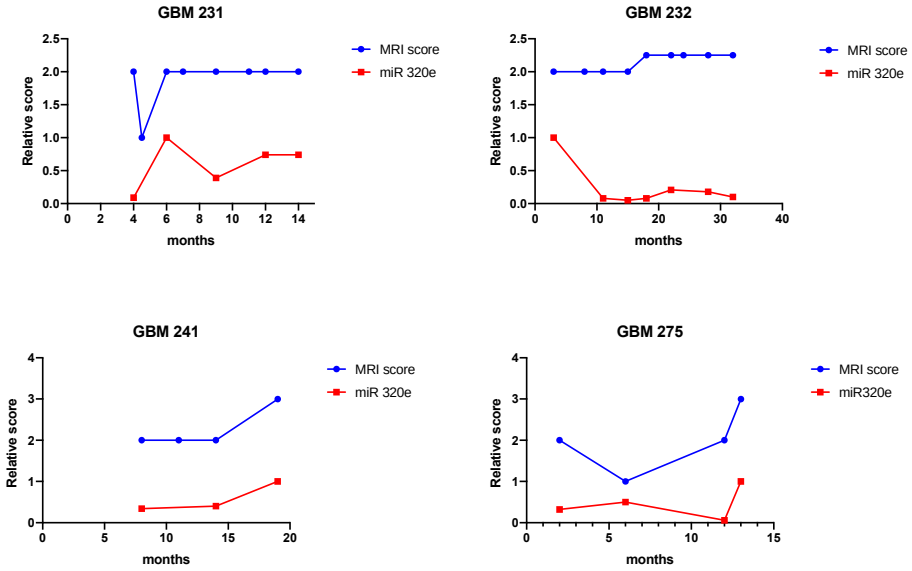

Fig 3. MiRNA-320e as a monitoring biomarker in glioblastoma. Diagrammatic timeline charts showing months on x-axis and relative miRNA concentration (orange) and MRI tumour volume (blue) on y-axis.
Arrows indicate timepoints of surgery.

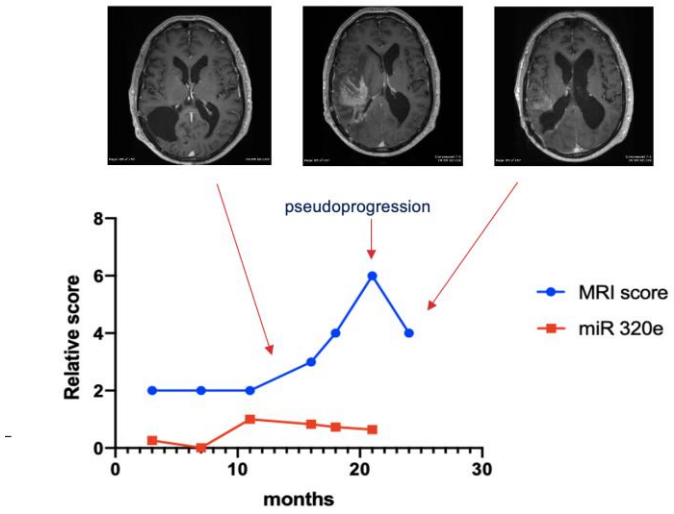

Fig 4. Example of GBM patient with pseudoprogression on MRI. Graph indicates stability of relative miRNA level despite rapidly increasing tumour size.

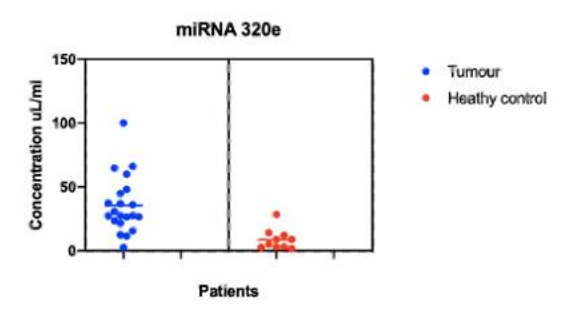

Fig 5. Concentration of miRNA 320e is higher in tumour patients compared to healthy controls

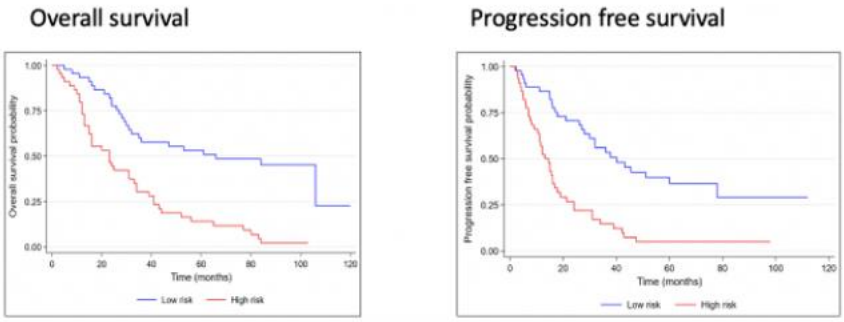

Fig 6. Kaplan-Meier curves demonstrating high (red) and low (blue) risk of progression divided by 6-miRNA gene signature.